Pond Dynamics/Aquaculture
Collaborative Research Support Program

NINTH WORK PLAN

for two-year investigations beginning between 1 August 1998 and 1 May 1999

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This work plan describes a standardized set of experiments to be undertaken by the Collaborative Research Support Program in Pond Dynamics/Aquaculture beginning between 1 August 1998 and 1 May 1999. Program activities are funded in part by Grant No. LAG-G-00-96-90015-00 from the United States Agency for International Development (USAID), Global Bureau, Office for Agriculture and Food Security. The authors' opinions expressed herein do not necessarily reflect the views of USAID.

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Contents of the Ninth Work Plan

Funded Ninth Work Plan Studies: Research Areas and Themes

		RESEARCH AREA: PRODUCTION OPTIMIZATION	
Pond Dynamics	Boyd	Pond Soil Characteristics	9PDR2
Feeds and Fertilizers	Lochmann	Fish Yields and Economic Benefits of Tilapia/*Clarias* Polyculture in Fertilized Ponds Receiving Commercial Feeds or Pelleted Agricultural By-Products	9FFR2
	Brown	Reduction of Rations below Satiation Levels	9FFR3
	Brown	Timing of the Onset of Supplemental Feeding	9FFR4
Reproduction Control	Fitzpatrick	Masculinization of Tilapia by Immersion in Trenbolone Acetate (a,b,& c)	9RCR5
	Phelps	Selection of Individuals for Sex Inheritance Characteristics for Use in Monosex Production	9RCR6
	Shelton	Monosex Tilapia Production through Androgenesis	9RCR7
Aquaculture Systems Modeling	Piedrahita	Model for Determining Aquaculture Pond Water Quality and Effluent Characteristics	9ASMR2
New Aquaculture Systems/New Species	Diana	Taro-Fish Culture in Ponds: Recycling of Pond Mud Nutrients	9NS1
	Diana	Culture of Mixed-Sex Nile Tilapia with Predatory Snakehead	9NS2
	Kohler	Spawning and Grow-Out of *Colossoma macropomum* and/or *Piaractus brachypomus* (a,b,c,d,& e)	9NS3
		RESEARCH AREA: ENVIRONMENTAL EFFECTS	
Effluents and Pollution	Wood	Use of Pond Effluents for Irrigation in an Integrated Crop/Aquaculture System	9ER1
	Fitzpatrick	Fate of Methyltestosterone in the Pond Environment (a,b,& c)	9ER2
	Diana	Integrated Recycle Systems for Catfish and Tilapia Culture	9ER3
Appropriate Technology	Bowman	On-Farm Trials: Evaluation of Alternative Aquaculture Technologies by Local Farmers in Kenya	9ATR1
		RESEARCH AREA: SOCIAL AND ECONOMIC ASPECTS	
Marketing and Economic Analysis	Engle	Development of Central American Markets for Tilapia Produced in the Region	9MEAR3
	Engle	Economic and Social Returns to Technology and Investment in Thailand	9MEAR4
	Hatch	Rapid Economic Evaluation Tools	9MEAR5
Adoption/Diffusion	Bowman	Aquaculture Training for Kenyan Fisheries Officers and University Students	9ADR3
	Bowman	Establishment of Companion Sites in the Africa Region	9ADR4
	Bowman	Regional Outreach in Africa	9ADR5
	Brown	Workshops and Production of Improved Extension Materials	9ADR6
Decision Support Systems	Bolte	Decision Support Systems for Fish Population Management and Scheduling in Commercial Pond Aquaculture Operations	9DSSR2
	Bolte	Enhancing the POND© Decision Support System for Economics, Education, and Extension	9DSSR3
		RESEARCH SUPPORT	
Database Management	Bolte	PD/A CRSP Central Database: Development and Management	9DM2
Education Development	McNamara	Building Research Capacity in CRSP Host Countries	9HCD2
	McNamara	Institutionalizing EDC Activities in CRSP Host Countries	9HCD3

INTRODUCTION

The Ninth Work Plan of the Pond Dynamics/Aquaculture CRSP was developed by the CRSP Technical Committee (TC) and describes activities to be conducted by the CRSP during the period from 1 August 1998 through 30 April 2001 under United States Agency for International Development (USAID) Grant No. LAG-G-00-96-90015-00. The start dates for Ninth Work Plan studies are staged between 1 August 1998 and 1 May 1999, with all final Ninth Work Plan reports due 30 April 2001 or earlier. (No one investigation extends beyond a two-year period although the overall time frame is greater than two years.) Previous activities under this grant are described in the Eighth Work Plan, which covered the period from 1 August 1996 to 31 July 1998.

Preparation of the Ninth Work Plan involved an unprecedented level of internal (within the PD/A CRSP) and external anonymous review of work plan proposals. Proposals were solicited based on priorities and constraints identified in the grant and in other PD/A CRSP documents as described in the Ninth Work Plan Request for Proposals. Well into the review and selection process, the PD/A CRSP learned of a substantial reduction in its USAID budget allocation. As a result, significant changes had to be made in the proposed work plan to ensure adequate coverage of the critical areas within the newly imposed financial constraints. A number of work plans were eliminated and research support activities curtailed. Additionally, substantial budgetary reductions were absorbed by the Program Management Office (PMO).

The goal of the CRSPs current five-year USAID grant, the *Continuation Plan 1996-2001*, is to provide a basis for improving the sustainability of aquaculture production systems. This approach uses two building blocks to identify research priorities: research in production systems; and capacity building via research support. Research in production systems is organized into three research areas (Production Optimization, Environmental Effects, and Social and Economic Aspects), which are further subdivided into specific research themes. The table on the facing page illustrates the distribution of Ninth Work Plan investigations among the three research areas, and further among research themes. CRSP research is assisted by research support functions which build capacity through educational development, and information and database management.

In relation to previous PD/A CRSP work plans, probably the most significant change is the elimination of the global experiment. Other differences from previous work plans are a new emphasis on regionalization of research and the extent of cross-cutting research projects. In the past, research plans were focused on individual countries, with limited efforts at extending the information to neighboring countries. In the Ninth Work Plan, processes and procedures are identified to ensure that information is disseminated to potential users beyond the borders of our host countries. Cross-cutting research encompasses projects that are conducted at several sites and in the US. Results from these projects are relevant to all host countries and the regions around them.

Work under the Ninth Work Plan will be implemented at the following prime sites: Peru, Kenya, the Philippines, and Thailand. Work in Honduras was interrupted at the conclusion of the Eighth Work Plan, and a request for proposals has been issued to identify a new lead institution for the Honduras work. In accordance with USAID guidelines, the new Honduras work will concentrate on freshwater aquaculture. The work plans for the Honduras project will appear in the Ninth Work Plan Addendum.

Beginning with the Ninth Work Plan, there will no longer be a procedure for requesting approval of work plan changes from the TC and PMO. Instead, researchers will provide notice of work plan changes by completing a form developed by the Technical Progress Subcommittee (TPS) of the TC to be submitted to the PMO along with other annual reporting elements. The information collected on the form will serve the TPS, the External Evaluation Panel, and others in evaluating progress on Ninth Work Plan projects. Project Leaders are still advised to contact the Management Entity if changes cause the research to differ significantly from that of the original approved work plan.

CROSS-CUTTING RESEARCH

In addition to specific research activities implemented at in-country sites in Central and South America, Africa, and Asia, the Ninth Work Plan includes activities that address cross-cutting themes which have more immediate, wider application than those from prime and companion site investigations. This research builds upon and expands information from earlier PD/A CRSP research. Work plans are presented for six research themes of cross-cutting research: Pond Dynamics; Reproduction Control; Aquaculture Systems Modeling; Effluents and Pollution; Marketing and Economic Analysis; and Decision Support Systems. Additional research themes—Feeds and Fertilizers; New Aquaculture Systems/New Species; Appropriate Technology; and Adoption/Diffusion—are addressed in regional research.

The dynamic interactions among nutrients and fish yield continue to be a major focus of the CRSP. The influence of pond sediments on pond dynamics will be addressed in proposed research. Pond soil samples from the PD/A CRSP sites will be characterized and related to the role in the cycling of chemicals used in aquaculture, including those that might impact the environment through effluents. This relates to chemicals such as steroids that are used in hatchery operations such as monosexing for reproductive control. Alternative methodologies to feeding hormones, such as immersion for more abbreviated periods of exposure, may reduce the overall amount of steroids used and thus released. Non-steroid techniques using chromosome manipulation might provide reproduction control without chemical use and thereby reduce the environmental impact. Also, chromosome manipulation can be used in conjunction with sex reversal to produce broodstock for monosex breeding programs, but for both of these applications, assumptions of the genetic basis of sex determination must be tested and verified.

The proposed work on aquaculture systems modeling will update a previously developed model that uses stochastic weather inputs. The updated model will be used to generate probability distributions for pond water quality and fish yields. Previous models developed under the PD/A CRSP have been programmed using a commercially available modeling language. While this strategy has simplified and speeded up the model development process, the resultant models are not particularly user-friendly and require special software to execute. The updated model to be developed under the Ninth Work Plan will be coded using conventional programming languages to produce a user-friendly, stand-alone application.

Social scientists' involvement in PD/A CRSP has increased over time, primarily studying localized issues. A broader approach will be used in the Ninth Work Plan by initiating studies to develop local tilapia markets and by developing a technique that can assist researchers to screen possible technologies for pond trials. Work will continue on the development of estimates of economic returns to the research investment of the PD/A CRSP. A beneficial aspect of these activities will be increased interaction among economists and site personnel.

Aquaculture managers are confronted with complex decisions for culture facilities, effects on the environment, and the role in larger farming systems. Analytical tools for decision support systems integrate knowledge from mathematical models, expert systems, and databases into software systems. CRSP researchers developed POND©, a software package that provides capabilities for simulation modeling and economic analyses of pond facilities. The software is based on fundamental principles of pond aquaculture. Several areas of improvement are being undertaken under the Ninth Work Plan, focusing on 1) improving the utility of POND© for education and extension purposes, 2) enhancing POND©s underlying models in the areas of pond sediment-water column interactions, 3) calibrating and validating POND© for additional culture organisms, particularly various shrimp species, and 4) enhancing POND©s ability to address scheduling and other applied pond management issues. These improvements should improve the usefulness of the software in addressing the needs of both educators and pond managers, and allow improved decision-making in areas related to fertilization, feeding, stocking, water use and effluent discharge, and economic optimization.

Pond Dynamics

Introduction

Relationships among pond bottom soils, water quality, and production of fish and shrimp have not been thoroughly elucidated. Therefore, a subproject within the PD/A CRSP is considering the role of bottom soils in pond dynamics. Although this research includes studies of the dynamics of soil organic matter and nutrient concentrations, its most important aspect is the acquisition of data on basic chemical and physical characteristics of pond soils at PD/A CRSP sites. These data will be useful to other investigators in evaluating the factors influencing production. They also will be used to develop a theory of pond soil development and to devise a pond soil classification scheme similar to the one used in terrestrial soil taxonomy.

Pond Soil Characteristics and Dynamics of Soil Organic Matter and Nutrients

Pond Dynamics Research 2/Study

Collaborating Institution
Auburn University
Claude E. Boyd
C. Wesley Wood

Objectives

1) To describe physical and chemical characteristics of the bottom soil profiles in ponds at freshwater PD/A CRSP sites (Philippines and Ayutthaya, Thailand) and at brackishwater shrimp ponds at Ranot, Thailand.
2) To measure changes in concentrations of carbon, nitrogen, and phosphorus in pond soils over time at PD/A CRSP sites (AIT, Thailand; Comayagua and Choluteca, Honduras; and Sagana, Kenya) and ascertain if soil respiration and availability of nitrogen and phosphorus to water are affected by these changes.

Significance

There has been relatively little work on the role of bottom soils in pond aquaculture (Boyd, 1995). For years, aquaculturists largely ignored bottom soils, but as production levels have increased, it has become apparent that bottom soils are an important factor in pond dynamics (Boyd, 1995; Boyd and Bowman, 1997). In recognition of the importance of pond soils, the PD/A CRSP Continuation Plan contains a bottom soil component in the pond dynamics research priorities.

The proposed work is a continuation of previous PD/A CRSP efforts in pond soil research. Bowman (1992) proposed a workable system of pond soil classification based on characteristics of the surface layer. Munsiri et al. (1995) found that pond soils quickly develop profiles such as those found in terrestrial soils. They suggested that these profiles could be used to improve pond soil classification by allowing the techniques of Soil Taxonomy (Soil Survey Staff, 1994) to be applied. However, in order to initiate the use of Soil Taxonomy techniques in pond soil classification, data on bottom soil profiles are needed for a diverse group of pond soils. So far, data have been collected for freshwater pond soil profiles in Alabama and Mississippi (Munsiri et al., 1995); PD/A CRSP sites in Abbassa, Egypt (former site); AIT campus, Bangkok, Thailand; Comayagua, Honduras; and Sagana, Kenya; and at the Brackishwater PD/A CRSP site in Choluteca, Honduras (Munsiri et al., 1996; Boyd et al., 1997). The PD/A CRSP project has already committed funding to obtain samples from the freshwater site in Iquitos, Peru, during the current work plan.

Organic matter and nutrient concentrations in pond soil profiles change over time (Tucker, 1985; Munsiri et al., 1995; Boyd, 1995), and these changes influence the availability of nitrogen and phosphorus in pond ecosystems (Masuda and Boyd, 1994; Boyd, 1995). Therefore, in addition to the effort on profiles and soil classification, samples are being collected annually at four sites (AIT campus, two sites in Honduras, and Kenya) to evaluate changes in organic matter and nutrient concentrations and availability over time. This effort has been funded for the current work plan.

The proposed research would provide enough additional information from pond soil profiles in different areas to initiate work on a pond soil classification scheme. The work also will show how changes in organic matter and nutrient concentrations in pond soil influence the availability of nitrogen and phosphorus.

Anticipated Benefits

This is a continuation of studies conducted on pond soil profiles, and when this work unit is complete, data will be available for pond soil profiles to include brackishwater and freshwater ponds from 12 sites of different soil characteristics representing four continents and seven countries. This will be sufficient data on soil profiles upon which to begin to integrate pond soils into the existing system of Soil

Taxonomy (Soil Survey Staff, 1994). At the completion of this work unit, there will be four years of data on changes in soil organic matter, nutrient concentrations and nutrient availability over time. The data also will be useful in providing a complete description of bottom soil characteristics at the Philippine and Ayutthaya, Thailand, PD/A CRSP sites. The soil classification system will be useful in predicting the limitations of pond soils in host countries and in pond aquaculture in general. The information on changes in nutrient availability over time and site soil characteristics will be especially useful on pond fertilization research and in the practice of pond fertilization.

Identification of Beneficiaries

The most important benefit of this study will be the development of a data base upon which to bring pond soils into the existing system of Soil Taxonomy. This will be of wide interest to researchers, extension agents, and fish and shrimp farmers. The information on changes in soil characteristics and nutrient availability over time is of general scientific interest, and small-scale farmers near PD/A CRSP sites and in neighboring countries will benefit from the findings through improvements in methods of pond fertilization. This study also will be valuable to solving soil-related problems in the US baitfish, channel catfish, and sportfish industries. One PI (C.E. Boyd) has soil projects funded by the Southern Regional Aquaculture Center in the US, and this study will complement that effort.

Collaborative Arrangements

This study will be implemented at PD/A CRSP sites through a collaborative effort of the US and host-country PD/A CRSP participants. The part of the work done on brackishwater ponds in Thailand will be done with the collaboration of the Dr. Prasert Munsiri, Director, Ranot Brackishwater Station, Thailand Department of Fisheries.

Study Design

Sites: Field research facilities at PD/A CRSP sites in the Philippines, Thailand, Honduras, and Kenya, and research ponds at the Ranot Brackishwater Station, Thailand.

Pond Facilities: Three (0.1-ha or size available) earthen ponds at each site.

Culture Period: Variable, but samples will be taken during the last month of the culture period if possible.

Stocking Rate: Variable, but we plan to use ponds stocked at fish biomass equivalent to about 1,000 kg/ha and shrimp ponds stocked at about 30 individuals/m^2.

Water Management: Water will be added to replace evaporation and seepage in freshwater ponds and 5% water exchange in brackishwater ponds.

Other Inputs: Nitrogen and phosphorus fertilizers will be applied to PD/A CRSP ponds at rates determined by the experiment in progress. Shrimp feed will be applied to brackishwater ponds at 3 to 5% of body weight per day. Lime inputs are acceptable.

Test Species: Nile tilapia (*Oreochromis niloticus*) in freshwater and black tiger prawn (*Penaeus monodon*) in brackishwater ponds.

Sampling Plan: Core samples will be taken by PIs from the Thailand sites in 1998 and from the Philippines site in 1999. The samples of surface soil for measuring changes in soil organic matter and nutrient concentrations over time will be taken in both years from the two Honduran sites and from the site in Kenya by resident investigators and shipped to Auburn University for analysis. The Project Leader will obtain the samples from Thailand for demonstrating changes over time during one of his visits to that country related to other business. He travels there at least twice per year. The soil cores for measuring characteristics of soil profiles will be taken with a 1-m-long, hand-operated, 5.0-cm-diameter core sampler (Wildco, Saginaw, Michigan) at water depths of 80-100 cm in each pond. Cores will extend through the soft sediment and include at least 10 cm of the original, undisturbed pond soil. Cores will be pressed upward with a plunger, cut into 2.0-cm-long segments with aid of a 2.0-cm piece of core tubing and a wide spatula (Masuda and Boyd, 1994). Segments will be processed for analysis as described by Munsiri et al. (1995). Core samples were taken from CRSP sites in Thailand and Honduras in early 1997, and analysis of data revealed that samples of the upper 4- to 5-cm layers of soil in ponds would be sufficient for detecting changes in soil chemical characteristics over time. The same ponds from which the initial cores were extracted in 1997 will be used to facilitate comparisons of data over time. The core sampler tubes will be used for obtaining the 4-cm-long core segments. Ten segments will be taken from each pond and combined to give one sample per pond. The samples will be dried at 60°C, and carried or shipped to Auburn University for analysis.

Core segments will be analyzed for bulk density, particle density, moisture content, particle-size distribution, color, pH, total phosphorus, dilute acid-extractable phosphorus, exchangeable cations (ammonia, calcium, magnesium, sodium, and potassium), and carbon. Analyses will follow standard protocol presented by Page et al. (1982) and Klute (1986). The analyses for moisture, bulk density, and color must be done on site. Other analyses will be made at Auburn University. The 4-cm-long core segments will be analyzed for particle-size distribution, pH, total phosphorus, acid-extractable phosphorus, sulfur, exchangeable cations (ammonium, calcium, magnesium, sodium, and potassium), and carbon.

Mineralization studies for carbon and nitrogen will be done on selected core segments. Mineralization studies will be conducted *in vitro* by placing 25 g of dry soil and 20 ml water in sealed chambers and trapping CO_2 evolved in alkali solution for measurement. Ammonia and nitrate in soil will be determined before and after incubation to determine rates of change. Aerobic incubation will be made for 30 days at 25°C, and anaerobic incubations will be made for 7 days at 25°C. Phosphorus dissolution studies will be done by placing 2-g samples of soil in 100 ml of distilled water and shaking to equilibrium phosphorus concentration on an oscillating shaker.

Statistical Methods and Hypotheses: The appropriate null hypotheses are as follows: Objective 1, soil physical and chemical characteristics do not change with soil depth; Objective 2, soil physical and chemical composition do not change over time in pond bottoms at any of the sites, and samples from all sites and for all years have the same rates of carbon and nitrogen mineralization and phosphorus solubility.

The data on soil profiles will be plotted as concentration of each soil variable versus depth. Depths will be grouped into layers to obtain increased homogeneity of variances (Kingery et al., 1994). Data will be analyzed as a split plot within each soil layer. Site will be the main plot, and soil depth within a layer will be the split plot. Ponds will be replications. Data from laboratory mineralization and microcosm studies will be compared statistically by t-tests.

Regional Integration

This project integrates well into the regional plan. The information obtained will be useful for identification of pond soils and for use in modifying pond fertilization methods for local conditions in the countries surrounding the PD/A CRSP sites.

Statement on Contribution to Region

This study will be 70% in Asia, 15% in Central America, and 15% in Africa.

Schedule

The core samples in Thailand will be collected in November or December 1998 and those in the Philippines will be taken in November or December 1999. Annual samples from Thailand, Honduras, and Kenya will be taken during July 1998, July 1999, and in April 2000.

Final Report

The final report will be submitted by 30 June 2000.

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Reproduction Control

Introduction

Reproduction Control remains one of the PD/A CRSP research priorities as part of the PD/A CRSP Continuation Plan 1996-2001, which identified broodstock and seed supply as a major constraint to the development of aquaculture. Reproduction control of tilapia—the species of focus for much of the CRSP research effort—is critical to the success of most forms of production because tilapia have such a high reproductive capacity. Tilapia are capable of reproducing at a small size, of breeding multiple times per year, and of caring for their young—all of these characteristics may lead to the production of too many young and/or undersize fish for market. To prevent tilapia from diverting energy required for growth to that required for reproduction, various management practices have been used, including the production of populations of fish that contain only one sex. Culture of such monosex populations of tilapia produced by treatment with steroids early in development has become common practice throughout the world. For the Ninth Work Plan, CRSP research will focus on measuring the potential limitations of this practice and on developing alternative methods for producing monosex populations of tilapia.

Feeding 17α-methyltestosterone (MT) to developing tilapia fry is an effective means of producing monosex populations (Green et al., 1997); nevertheless, alternative methods require investigation because of the concerns raised about production of steroid wastes and metabolites that are potential environmental contaminants. While Ninth Work Plan Effluents and Pollution studies examine the effects of MT-feeding on pond soils, alternatives to feeding with MT are also being examined. Other methods for masculinization of tilapia such as immersion in steroid-containing solutions will be examined after promising results from previous CRSP research (Gale et al., 1995; Contreras-Sanchez et al., 1997). Development of techniques for masculinization through immersion may provide aquaculturists with a safe and cost-effective alternative to treating fry with food that contains MT.

Another approach to producing monosex populations of tilapia involves the use of chromosome manipulation techniques (Thorgaard and Allen, 1986). In the Eighth Work Plan, studies were initiated to develop methods for inducing androgenesis in tilapia. Such animals have only paternal genetic material because the maternal genome has been destroyed by ultraviolet radiation. By having only paternal genetic material, androgenetic tilapia should have two possible sex chromosome configurations: XX (female) or YY (male). These latter animals should theoretically sire only male offspring when mated to normal females, and thus would be valuable for use in producing monosex male populations for culture without treatment with steroid. In the Ninth Work Plan, studies are proposed to continue development of androgenetic techniques—namely, to use the irradiation protocol developed in the Eighth Work Plan in experiments designed to establish subsequent treatment conditions that will return the fish to the normal diploid complement of chromosomes and thereby optimize survival.

The successful development of androgenetic techniques for production of monosex populations of tilapia relies on the assumption that sex determination in this species is based on inheritance of sex chromosomes. However, Eighth Work Plan studies confirmed and extended earlier work (Shelton et al., 1983) that the sex ratios of Nile tilapia populations can deviate significantly from the expected 1:1 Mendelian ratio, suggesting that sex determination in Nile tilapia may involve genetic determinants on sex chromosomes and other chromosomes (autosomes). Crosses of tilapia that result in sex ratios deviating severely from 1:1 males to females may indicate one or both parents with excessive autosomal influence on sex determination. Such autosomal influence may explain how some YY males sire populations with sex ratios well below 100% male (Mair et al., 1995). Perhaps only YY males bred with females that have minimal autosomal influence on sex determination will result in 100% male offspring. Therefore, in studies proposed for the Ninth Work Plan, females without such excessive autosomal influence will be identified by progeny testing of repeated crosses. Those females that always produce 1:1 sex ratios will then be used in crosses with androgenetically-produced YY males. In addition, the performance of all-male progeny of such crosses will be compared side-by-side with that of MT-masculinized males at a variety of CRSP sites.

REPRODUCTION CONTROL

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Masculinization of Tilapia by Immersion in Trenbolone Acetate

Reproduction Control Research 5 (9RCR5)/Experiment, Experiment, Study

Collaborating Institutions

Oregon State University
Martin Fitzpatrick
Carl Schreck

Objectives

1) To determine if a single immersion of tilapia fry in trenbolone acetate results in masculinization.
2) To determine the growth performance of trenbolone acetate-immersed tilapia.
3) To determine how long trenbolone acetate persists in the water and treatment container.

Significance

Masculinization of tilapia fry by dietary treatment with anabolic steroids such as 17α-methyltestosterone (MT) is common in aquaculture (Green et al., 1997); however, other methods for masculinization of tilapia such as immersion in steroid-containing solutions are not well developed. In CRSP experiments conducted for the Eighth Work Plan, MT feeding was shown to result in the persistence of MT in soil for at least several weeks after the end of treatment. Such MT in the pond environment may pose a health risk to workers and exposure risk to other organisms, including fish, in the pond environment.

The use of immersion technology has the advantage that the steroid solution in contained within the treatment vessel, and thus can be easily processed. In previous CRSP experiments, Gale et al. (1995) demonstrated that two 3-hr immersions of tilapia fry in a solution containing 17α-methyldihydrotestosterone (MDHT) resulted in masculinized populations—a significant breakthrough in the development of short-term immersion technology for tilapia. In the Eighth Work Plan, we found that masculinization of tilapia can be achieved by a single 2-hr immersion in either MDHT or trenbolone acetate; however, single immersions have not achieved greater than 90% male populations (the minimum considered necessary to achieve the benefits of growing masculinized populations) which suggests that better results can be attained by improving treatment timing or dose. The proposed work will examine if single immersion in trenbolone acetate can result in greater than 90% masculinization. In addition, the fate of trenbolone acetate in the treatment container will be determined by measuring its persistence in water. Trenbolone acetate has been selected because of recent favorable statements from the Food and Drug Administration regarding its use in aquaculture.

Anticipated Benefits

Development of techniques for masculinization through immersion in trenbolone acetate-containing solutions may provide aquaculturists with a safe and cost effective alternative to treating fry with food that contains MT, because immersion will require substantially shorter exposure periods and the steroid will be contained for controlled filtration or biodegradation.

Experimental Design

Previous experiments in our laboratory have demonstrated that the percentage of male tilapia produced by immersion in trenbolone acetate was 90% if treated on Days 10 and 13 post-fertilization, and 80% if treated on Day 13, but there was no significant masculinization if they were treated on Day 10. Therefore, to address Objective 1, an experiment will be conducted to determine if a single immersion in trenbolone acetate at 12, 13, 14, or 15 days post-fertilization is as effective for masculinization as the two day exposure or feeding MT. Based on the results of this experiment, a second experiment will examine the level of masculinization achieved by a single immersion on the most effective day in 100, 500, and 1000 µg/L of trenbolone acetate. To address the second objective, the most effective treatment conditions (day and dose) will be used to masculinize tilapia fry and then after removal of the fry, the water will be sampled at 1, 2, 4, and 7 days (and every 7 days thereafter until disappearance) after treatment for measurement of trenbolone acetate.

Experiment A: Effect of treatment timing and dose on masculinization with trenbolone acetate
Site: Oregon State University, Corvallis, OR

Laboratory Facilities: Oregon State University—two aquaria containing a total of two males and six females for production of fry, 3.7 L chambers (containing 3 L of dechlorinated oxygenated water for steroid treatment), 75 L tanks for grow-out.

Culture Period: 60 days for offspring.

Stocking Rate: 100 fry/container.

Water Management: Water temperature will be maintained at 28-30°C.

Other Inputs: none

Test Species: Nile tilapia (*Oreochromis niloticus*), Ivory Coast strain.

Sampling Plan: The first phase of the experiment consists of groups of fry immersed in trenbolone acetate at 500 µg/L for 3 hr on 12, 13, 14, or 15 days post-fertilization (dpf), or fry immersed in ethanol vehicle (previous experiments have shown that there is no difference in the sex ratios of fish exposed to the ethanol vehicle and untreated fry). All treatments will be triplicated. On the treatment date, the fry will be carefully netted from their container and then placed in a new container that contains either trenbolone acetate dissolved in ethanol or the ethanol vehicle (Days 12 and 15 only). After treatment, the fry will be returned to clean water. After one week, the fry will be transferred to the grow-out tanks.

In the second phase of the experiment, groups of fry will be immersed on the optimal masculinization day (determined in the first phase) in trenbolone acetate at 100, 250, 500, or 750 µg/L, or ethanol vehicle for 3 hr. All treatments will be triplicated. On the treatment date, fry will be carefully netted and then placed in a separate container that contains either trenbolone acetate dissolved in ethanol or the ethanol vehicle. After treatment, the fry will be returned to clean water. After one week in the jars, the fry will be transferred to the grow-out tanks.

Water quality parameters will be measured daily (pH, temperature) or weekly (ammonia, nitrates, and dissolved oxygen). At 60 dpf, the tilapia will be killed to determine the sex ratios.

Statistical Methods and Hypotheses: $H_0 1$: Sex ratios of tilapia in groups treated with a single immersion in trenbolone acetate do not differ from controls. $H_0 2$: Sex ratios of tilapia do not differ between groups immersed in trenbolone acetate on different days. $H_0 3$: Sex ratios of tilapia do not differ between groups immersed in trenbolone acetate at different doses. Sex ratios will be compared between MT-fed and control groups by Chi-squared test.

Deliverables: A technical report and possibly a journal article.

Schedule: Data collection, 9/98-1/99; technical report, 7/99.

Experiment B: Growth performance of trenbolone acetate-immersed tilapia
Site: Oregon State University, Corvallis, OR

Laboratory Facilities: Oregon State University—two aquaria containing a total of two males and six females for production of fry, 3.7 L chambers (containing 3 L of dechlorinated oxygenated water for steroid treatment), 75 L tanks for grow-out.

Culture Period: 180 days for offspring.

Stocking Rate: 100 fry/container.

Water Management: Water temperature will be maintained at 28-30°C.

Other Inputs: none

Test Species: Nile tilapia (*Oreochromis niloticus*), Ivory Coast strain.

Sampling Plan: Based on the results of experiment 1, groups of fry will immersed in trenbolone acetate at the optimal time and concentration, fed MT at 60 mg/kg food for 28 days beginning at the onset of feeding, or left untreated (a preliminary experiment will determine if untreated fish grow comparably to fish immersed in ethanol vehicle or fed with ethanol-treated food). Each treatment and control group will be triplicated. After 28 days in the start-up containers, all groups will be transferred to 75 L tanks and grown for another 5 months. Every month, 20 fish from each tank will be anesthetized, weighed, measured for length, and then returned to the rearing tanks (densities will be equalized to adjust for mortality). At the end of 6 months, all fish will be sampled for weight, length, and sex.

Water quality parameters will be measured daily (pH, temperature) or weekly (ammonia, nitrates, and dissolved oxygen).

Statistical Methods and Hypotheses: $H_0$1: There is no difference between treatment groups in the growth rate of tilapia. $H_0$2: Sex ratios of tilapia do not differ between groups. Weight and length data will be compared between treatment and control groups by analysis of variance. Sex ratios will be compared between groups by Chi-squared test.

Deliverables: A technical report and possibly a journal article.

Schedule: Data collection, 4/99-10/99; technical report, 12/99.

<u>Study C: Detection of trenbolone acetate in water after treatment</u>
Site: Oregon State University, Corvallis, OR

Laboratory Facilities: Oregon State University—two aquaria containing a total of two males and six females for production of fry, 3.7 L chambers (containing 3 L of dechlorinated oxygenated water; Waters 660E Multisolvent Delivery HPLC system for separation and detection (by ultraviolet light absorption) of trenbolone acetate.

Culture Period: 180 days for offspring.

Stocking Rate: 100 fry/container.

Water Management: Water temperature will be maintained at 28-30°C.

Other Inputs: none

Test Species: Nile tilapia (*Oreochromis niloticus*), Ivory Coast strain.

Sampling Plan: The experiment consists of two groups: 1) fry immersed at the optimal masculinization conditions established in experiments 1 and 2; and 2) fry immersed in ethanol vehicle. Each group will be triplicated. Water samples (25 ml) will be collected on 0 (before and after treatment), 1, 2, 4, and 7 days (and every 7 days thereafter until disappearance) after treatment for measurement of trenbolone acetate. All samples will be extracted on Sep-Pak and then analyzed for presence of trenbolone acetate and metabolites by HPLC At the end of the 6-month grow-out period, the tilapia will be killed to determine if the treatment with trenbolone acetate resulted in masculinization.

Statistical Methods and Hypotheses: $H_0$1: Trenbolone acetate is non-detectable in water during or after immersion treatment of tilapia fry. This is a descriptive study and therefore, statistical analysis is unnecessary for testing the null hypothesis. Sex ratios will be compared between MT-fed and control groups by Chi-squared test.

Deliverables: A technical report and possibly a journal article.

Schedule: Data collection, 10/99-3/00; technical report, 7/00.

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Selection of Individuals for Sex Inheritance Characteristics for Use in Monosex Production*

Reproduction Control Research 6 (9RCR6)/Study

Collaborating Institution
Auburn University
Ron Phelps

Objective
To develop lines of Nile tilapia, *Oreochromis niloticus*, that will breed true for a given sex ratio.

Significance
There is evidence that in domestic stocks of *O. niloticus*, sex ratios of individual pairs may vary from 50:50. Shelton et al. (1983) found that 21% of the pair spawns were outside a 95% confidence interval for an expected 50:50 ratio. Karsina (1993) found that 32% of 22 pair spawns did not conform to an expected 50:50 ratio. Lester et al. (1989) found sex ratios from 60 families to range from 2 to 60% male. Abucay (1997) reported sex ratios ranging from approximately 30 to 70% males from presumed XX females x XY males. Baroiller et al. (1993) mated hormone-induced "XX" male O. *niloticus* to normal XX females. The resultant groups of progeny should have been all female, but for fry held at 27 to 29°C they found that 26% of the spawns contained > 10% males and when at 32 to 36°C, 92% of the spawns had > 10% males.

This variability in sex ratios is a challenge in the development of YY breeding programs. Mair et al. (1995) reported that YY males gave a mean of 96.5% male offspring but with a range of 67 to 100%. In the culture of large tilapia, populations of > 95% males are desirable and any variability less than that can impact the quantity of marketable fish. Lovshin et al. (1990) found that even 5% females in a population reduce adult production by almost half after nine months of culture. For YY production of males to be successful, variability in the percent males produced must be reduced and parent lines that conform to a simple Mendelian inheritance established.

It may be possible to select for individuals which consistently produce progeny with a 50:50 sex ratio. Baroiller et al. (1993) found that some pairs of *O. niloticus* gave progeny whose sex ratios were influenced by temperature while other pairs were not. Mair et al. (1990) also found some individual pairs which gave progeny that were not influenced by temperature. Wohlfarth and Wedekind (1991) concluded that sex ratio is a heritable trait and responds to selection. They reported that progeny from individuals giving a skewed sex ratio would also produce a skewed ratio while those from fish giving a 50:50 ratio have ratios similar to their parents. Repeated spawns of a single pair have been consistent in the sex ratios of the progeny (Shelton et al., 1983; Wohlfarth and Wedekind, 1991). It is then possible to select for lines of *O. niloticus* that will give consistent sex ratios near 50:50. The procedure of androgenesis should eliminate any cytoplasmic effects on sex ratios and when the procedure is applied to fish that give consistent sex ratios then the possibility of all-male seed production is greatly enhanced. The combination of androgenically produced YY males from true breeding parent stock crossed with true breeding females should give the consistent results needed for YY male technology to become widely adopted. But before YY male technology can be advanced, true breeding lines of tilapia based on the results of individual pair spawns must be established.

Experimental Design
Site: Auburn University in collaboration with the University of Oklahoma

Study Period: July 1, 1998 to June 30, 1999

Facilities: A minimum of 30 aquaria and 35 hapas would be utilized at Auburn University for the proposed activity.

* As of the time of printing, the PMO has approved this investigation for funding, but the formal subcontract for work is still in process.

Plan of Work: Selected males and females from two or more strains, which the sex ratios of the progeny are know, will be mated in an attempt to obtain target sex ratios. These fish will have been identified as part of the Eighth Work Plan. Individuals which have given progeny conforming to 50:50 ratio will be mated and the sex ratio of the progeny determined. Attempts will be made to obtain a minimum of three spawns from each pair. Half of the progeny from each spawn will be held at 27 to 30°C and half at 36°C for 30 days during the period of gonadal differentiation to determine if the sex ratio from a given pair spawn is susceptible to environmental influence.

Progeny of selected spawns will be reared to maturity and mated to other families of similar sex ratio history and the sex ratio of the progeny determined. Families which give repeated spawns not differing from a 50:50 sex ratio and are not susceptible to temperature effects on sex ratio will be held for mating with YY males.

Statistical Design: Null Hypothesis: Sex ratio is not an inheritable characteristic. Sex ratio data will be analyzed by Chi square. The effects of temperature on sex ratio be analyzed by ANOVA.

Regional Integration

All regional programs except one are using monosex tilapia as a basic part of their program and would benefit from the knowledge gained from the proposed work. This study is a precursor to a direct collaboration with three of the country programs. It is linked to the Kenyan program in the establishment of sex ratios of selected strains of *O. niloticus*.

Schedule

The study period is 1 May 1999 to 30 April 2001. Field activities will take place from 7/1/99 to 10/1/99 and from 7/1/00 to 10/1/00. Sex ratios will be determined and data analyzed from November 1999 to March 2000 and from November 2000 to March 2001. A progress report will be submitted by 4/30/00, and a final report by 4/30/01.

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Monosex Tilapia Production through Androgenesis*

Reproduction Control Research 7 (9RCR7)/Experiment

Collaborating Institution
University of Oklahoma
William Shelton

Objective
To increase the overall sustainability of aquacultural systems through production optimization by developing short- and long-term solutions to reproduction technology problems. Specifically, use androgenetic procedures to develop direct induction of YY-male Nile tilapia, *Oreochromis niloticus*, for the production of all-male offspring.

Significance
One of the major constraints to aquaculture development is the reliability and sustainability of seed organisms for culture. Within the context of tilapia farming, this limitation involves effective and practical control of unmanaged recruitment. Various approaches to monosexing fishes have been applied to culture, but the use of chromosome manipulation is one of the more recently implemented practices (Shelton, 1989; Dunham, 1990). Gynogenesis, which is the production of progeny with only a maternal genome has been investigated widely, but the development of techniques to induce androgenesis, i.e., progeny with only a paternal genome, is much less studied (Thorgaard and Allen, 1986). Androgenesis can provide a mechanism for the development of unique broodstock to use in producing all-male progeny without the use of sex-reversing steroids. Within the contemporary atmosphere of increasing governmental regulation on use of chemicals on food fish, continued dependency on steroid-induced monosexing places the culture of tilapias in a precarious position. The recently developed protocol to develop YY-male tilapia relies on estrogen treatment, and even though fish to be cultured are one generation removed from the treatment the protocol still depends on progeny testing to identify target broodstock (Scott et al., 1989; Mair et al., 1997). In contrast, androgenesis offers the potential of direct induction for YY-male tilapia, without chemical treatment, and without the need for progeny testing to identify the unique male broodstock. Although the system is based on incompletely characterized genetics of sex determination (Wohlfarth and Wedekind, 1991; Mair, 1993), the perceived complications might be minimized by using select strains. Sex determination is characterized by a homogametic/heterogametic genotype; however, autosomal modifier genes may alter the theoretical 1:1 progeny sex ratio (Shelton et al., 1983), therefore, deviations in the expected all-male progeny from YY-male breeding should be anticipated. Thus, this study will involve not only an investigation of a protocol to produce androgenotes (progeny with only paternal genome), but also examination of the basic mechanism of sex determination. Androgenesis should result in offspring of equal sex ratio; females will be XX, but males will be YY. Progeny testing will be required during experimental development to confirm that the males are fertile and that only male offspring (XY) will result when spawned with normal females (XX). Sex ratio of progeny from crosses of androgenote females and YY-male androgenotes (a full sibling cross) will be compared with outcrosses of YY-males with females from other stocks. This will provide a basis to collaborate with the PD/A CRSP investigators at Auburn University on their study of strain variations in sex ratio inheritance (Eighth Work Plan). Ouputs from our study will be the number of androgenotes (males and females); each will be progeny tested as a component of examining the genetic basis of sex determination. The YY-males will be the foundation for developing a unique broodstock that will produce all-male progeny, and will provide insight into the expected variation in the sex determining system.

Anticipated Benefits
Tilapia culture is one of the fastest growing forms of finfish aquaculture in the world. Production efficiency and product marketability in most economies depend on the capacity to control unwanted reproduction. Techniques for managing recruitment have evolved from traditional means to the current

* As of the time of printing, the PMO has approved this investigation for funding, but the formal subcontract for work is still in process.

practice of steroid-induced sex reversal, which has been the industry standard for monosexing during the last couple of decades. However, chemical treatment of food fish has become increasingly constrained, and since this monosexing tool could be withdrawn, an alternative should be available. The monosex breeding program using YY-male broodstock might provide such a program. Through androgenesis, YY-males can be produced directly without the use of steroids, and without the need for progeny-test identification.

Research Design

Site: University of Oklahoma in collaboration with Auburn University. Techniques for androgenesis in tilapia will continue to be researched at the University of Oklahoma. The experiment will be an extension of Study II and the initiation of Study III. The latter was discussed as a component of the total program chronology, but not one that could be completed within the two years of the Eighth Work Plan of the PD/A CRSP Continuation Plan 1996-2001.

Plan: Techniques developed during year one of the Eighth Work Plan (Shelton, in review) will be incorporated in the protocol for the experiment to be done under the Ninth Work Plan. The developments include managed aquarium spawning (Rothbard and Pruginin, 1975; Yeheskel and Avtalion, 1986; Myers and Hershberger, 1991), incubation of artificially propagated zygotes (Rana, 1986) and application of Developmental Rate (mitotic interval - τ_0) (Dettlaff and Dettlaff, 1961) to diploidization (shock) protocol (Shelton and Rothbard, 1993; Shelton et al., 1997). Induction of androgenesis for the Nile tilapia must focus on two components within the chromosome manipulation regimen: (1) Gamete (egg) treatment with Ultra-Violet (UV) to deactivate the maternal DNA, and (2) Diploidization of activated haploid paternal zygotes through endomitotic shock treatment.

Optimization of UV treatment. Freshly ovulated eggs will be collected from Nile tilapia. Separate groups of eggs will be exposed to UV irradiation using a UV-crosslinker. Dosage will range from 0 (control) to 1,000 Joules/m^2 (J/m^2) in increments of 100. Maternal DNA in tilapia eggs is reported to be dimerized between 200 and 600 J/m^2 (Myers et al., 1995). Ova will be activated with conspecific spermatozoa and incubated to hatching. Hatch rate will decrease with increasing UV dose and zero hatch will indicate complete female genome deactivation. Haploids may develop to hatch, but they will be recognizable by various malformations. Trials with several females will be required to determine the range of UV dose which will dependably destroy the genome. Progress is expected on this component during the second year of study II of the Eighth Work Plan; the developed UV treatment will be incorporated into androgenetic protocol.

Diploidization through endomitotic shock. Optimization of shock protocol will require testing the combination of several parameters. Thermal shocks will be tested because of the ease of application and its effectiveness in diploidizing by either polar body (early) or endomitotic (late) shock. The use of pressure shock may be comparatively examined, but only after thorough evaluation of thermal trials. Both cold shock and heat shock have been effective (Don, 1989; Hussain et al., 1993). We will compare the two types of thermal shock based on chromosome manipulation information from the literature; magnitude and duration have been optimized, but the times of application for heat and cold shock in these earlier studies are not in agreement (Don, 1989; Mair, 1993; Myers et al., 1995). This is the rationale for referencing shock administration in tau units and for the comparison of shock types. Heat shock will be applied at 42.5°C and cold shock will be applied at 11°C; the duration of heat will be 3.5 min while the cold application will be for 60 min. Paired tests will compare induction in UV-treated eggs from individual females. Time of initiation should coincide with metaphase of the first mitosis, which is temperature dependent (Saat, 1993). Pre-shock incubation temperature will be controlled, but diploidization rate will be related to tau-units as well as in absolute time. Shock time (τ_s- minutes) expressed in terms of Developmental Rate (τ_0) or τ_s / τ_0 will provide a dimensionless treatment index which will permit adjustment of absolute shock time with reference to different incubation temperatures.

Broodstock for diploidization studies will have a genetic marker as a treatment control. The eggs from a color mutant (red) of the Egyptian Nile tilapia strain will be used in the androgenetic studies (McAndrew et al., 1988). This color is controlled by a homozygous dominant allele. Normal color males will be used. Progeny from a control cross will be of the red phenotype, while diploid androgenotes will be normal color like the male parent. Any progeny with the red phenotype will be discarded. Offspring should include females and males in a 1:1 sex ratio, but the male genotype is expected to be YY.

Progeny from androgenote males are expected to be only male (XY), but this must be verified to demonstrate the viability of the YY-genotype, and to test variability in sex determination. One component of the progeny test will be full-sibling crosses, i.e. androgenote males with androgenote females (Table 1); outcrosses with females from other families or strains will be compared to the sib-matings. This phase will address the collaboration with the Auburn studies and provide a basis for comparing genetic variation in sex determination. Stocks tested at Auburn which have been identified as having progeny that most closely conform to the expected sex ratio of 1:1 will be logical broodstock for the outcross tests.

Table 1. Expected progeny sex ratios from crosses of androgenotes (full-siblings) and outcross males and females

	XY (normal male)	YY (androgenote)
XX (normal female)	1:1 (control)*	0:1 (outcross)
XX (androgenote female)	1:1 (outcross)	0:1 (sib-cross)

* female: male sex ratio

Regional Integration

The output of androgenotes and progeny tests will integrate studies at Auburn on strain testing. The testing at Auburn also integrates with the East Africa Work Plan.

Schedule

Experiments were started under the Eighth Work Plan. Optimization of androgenesis will continue through the end of the program (2001). Study II of the Eighth Work Plan began June 1997; results will be used in the present proposed study; a progress report for androgenesis will be provided in July 1999 and for progeny testing in July 2000. A final report will be provided by 30 April 2001.

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AQUACULTURE SYSTEMS MODELING

Introduction

Modeling of aquaculture pond systems has been a continuing activity of the PD/A CRSP. Over the years models have been developed to simulate water quality parameters under a variety of conditions. Initial models were built under the assumption that pond conditions were approximately uniform. Later on, models were developed such that stratification events could be simulated over short periods of time. Those models were further refined so that long-term simulations of up to a full growing season could be carried out. In doing these long-term simulations, the models were enhanced to account for stochastic variations in weather. Other models have been developed to look at nutrient cycling in integrated aquaculture–agriculture systems. All the models developed to date at UC Davis have been implemented using STELLA™, a commercially available modeling language available for both Macintosh and Windows operating systems. Although the use of STELLA™ for model development greatly simplified and accelerated the process of model programming, it has certain limitations for model distribution and use by interested individuals. Under the work proposed for the Ninth Work Plan, a PD/A CRSP model previously developed at UC Davis will be updated and re-coded to create a "user-friendly" version that can be run without specialized programming knowledge or software packages.

Model for Determining Aquaculture Pond Water Quality and Effluent Characteristics*

Aquaculture Systems Modeling Research 2 (9ASMR2)/Study

Collaborating Institution
University of California, Davis
Raul H. Piedrahita
Zhimin Lu

Introduction
The work being proposed here expands on models previously developed at UC Davis. The objective in this work plan is to enhance the usefulness of previous models by improving the accessibility and realism of pond water quality, fish yield, and effluent simulations. Nutrient, especially nitrogen and organic matter, dynamics will be considered in a model which also integrates stochastic features. In a shift from previous models developed at UC Davis, the new model will be specifically developed with a user-friendly interface, and in a form that can be easily distributed to users.

The work being proposed under this work plan complements work by the OSU Biosystems Analysis Group. The model to be developed at UC Davis will have user interfaces developed in consultation with the OSU Biosystems Analysis Group in an attempt to make those interfaces compatible with the POND© interfaces. The UC Davis model will emphasize characteristics such as stochastic generation of weather parameters, stratification, and detailed nutrient cycles. The UC Davis model will also have as an objective the evaluation of potential environmental impacts by analyzing water and nutrient releases as affected by environmental conditions and by pond management practices.

Objectives
The specific objectives of the proposed work are in accordance with the objective listed in the Continuation Plan for the Aquaculture Systems Modeling Research Theme: "To analyze and synthesize research results into models which better describe system processes." The specific objectives are:

1) To develop a stochastic model for estimating water quality and fish growth in fish ponds as determined by climate, pond management practices (feeding and fertilization), and quantity and quality of the water supply. The model will be of use in obtaining probability distributions for estimates of water quality, fish yield, and nutrient and water releases from ponds.
2) To program the model in a form that is "user-friendly" and can be run without specialized programming knowledge, or software packages.

In addition to supporting the Aquaculture Systems Modeling Research Theme, the objectives also support the Effluents and the Appropriate Technology Research Themes.

Significance
The development of models to predict fish yields and effluent water quality from aquaculture operations is an important tool in the process of improving the sustainability of aquaculture (Briggs and Funge-Smith, 1994). The models can be run with climatic and other site-specific data in addition to pond management information, to determine the potential aquaculture yields, and nutrient and water discharges. Stated objectives of the Continuation Plan include the reduction of effluents and the development of appropriate technologies, and the use of a model such as the one being proposed here can result in progress towards those objectives.

The potential impact that aquaculture may have on the environment has received considerable interest recently by scientists, regulators, and aquaculturists (Briggs and Funge-Smith, 1994; Schwartz and Boyd, 1994; Kelly et al., 1994; Hopkins et al., 1993). Furthermore, previously aquaculture had an image as an environmentally benign practice but this has changed in recent times. At the 1997 meeting of the

* As of the time of printing, the PMO has approved this investigation for funding, but the formal subcontract for work is still in process.

World Aquaculture Society in Seattle, there were demonstrations and "counter conferences" organized by a variety of activist environmental groups. The increased interest in the environmental impacts of aquaculture has been due, in large measure, to the rapid growth of aquaculture and the environmental degradation that has occurred in mangrove areas and in estuaries through shrimp farming and other aquaculture practices (Pillay, 1992). Effluent discharge from aquaculture may have deleterious effects on the biota in receiving waters through oxygen depletion, increased suspended solids concentration, temperature changes, diseases, and eutrophication (Ackefors and Enell, 1994). Application of aquaculture effluents to agricultural land can be used to enhance the growth of crops. Land application of aquaculture effluents can also reduce the environmental impact of discharged effluents by removing suspended solids and nutrients. Salt concentrations in some aquaculture waters and scheduling problems in matching the irrigation and nutrient needs of the terrestrial crop with the availability of aquaculture effluents are some of the difficulties encountered in using aquaculture effluents in agriculture.

Modeling work being conducted at UC Davis under the Eighth Work Plan has shown that organic matter and nitrogen dynamics in the sediment and water column of waste-fed aquaculture ponds can be reliably predicted using mechanistic models. In other work at UC Davis, it has been shown that temperature, dissolved oxygen, and fish growth can be predicted using stochastic methods. The work being proposed for the Ninth Work Plan will involve the linking of the stochastic temperature and DO model with the water column and sediment organic matter/nitrogen dynamics model. However, previous models developed by the UC Davis DAST have been programmed using STELLA™, a specialized modeling language. Whereas the use of STELLA™ has greatly facilitated the model development process, the resulting models are not in a form that can be easily distributed and used by non-modelers. Therefore, the combined and enhanced stochastic water quality and fish yield model will be coded using a programming language such as C++ so that user-friendly interfaces can be incorporated, and the model can be distributed for execution without the need for specialized software.

Anticipated Benefits

Much of the modeling work carried out at UC Davis has received relatively little exposure and use outside the realm of researchers and modelers. In this work plan, we intend to integrate our past efforts in the development of a stochastic model, and of the detailed nutrient cycling included in the pond model that is part of the coupled aquaculture/agriculture systems model. Furthermore, the final version of the model developed under the Ninth Work Plan will summarize the mechanistic models of aquaculture ponds developed at UC Davis in a user-friendly and accessible tool. The new modeling tool will be designed to be used to assess carrying capacity, potential fish yields, and effluent flow rates and characteristics at a given site. The target users for the new model will be primarily researchers, designers and aquacultural development planners.

Activity Plan

The work to be carried out under the Ninth Work Plan has two major components. One component is the integration and enhancement of models previously developed at UC Davis. The second component is the re-formulation of the combined model into a form that is easily distributed and used by non-modelers. Details of the research plan are presented below.

Location of Work: All programming work will be carried out at UC Davis. A one week annual working visit to Corvallis will take place. The meeting will be used to coordinate the work with the OSU Biosystems Analysis Group.

Research Plan and Methodology: During the Eighth Work Plan, and under the general heading of Aquaculture Pond Modeling for the Analysis of Environmental Impacts and Integration with Agriculture, the UC Davis group has been working on two studies: ASMR1) Relationship Between Carbon Input and Sediment Quality in Aquaculture Ponds, and ASMR2) Stochastic Modeling of Temperature, Dissolved Oxygen and Fish Growth Rate in Aquaculture Ponds. The work to be undertaken in the Ninth Work Plan will have two distinct components related to the specific objectives listed above:

1) *To develop a stochastic model for estimating water quality and fish growth in fish ponds as determined by climate, pond management practices (feeding and fertilization), and quantity and quality of the water supply.* The two models developed under the Eighth Work Plan will be combined into a single model. In the first Study under the Eighth Work Plan, a model has been developed to simulate the flow of nitrogen and of organic matter between an aquaculture pond and a coupled terrestrial crop system. Although the model includes sediment/water interactions, this is an area for which there is very limited information available. Field work currently in progress under the PD/A CRSP Eighth Work Plan will provide information which will complement and enhance previous knowledge on pond sediments and how they interact with the water column. This information will be used to update the simulation of sediment/water column interactions.

   The new, integrated model will have the detailed water column/sediment interactions and nutrient relationships included in the Eighth Work Plan Study ASMR1 model, as well as the stochastic weather generation and solution procedures developed for the Eighth Work Plan, Study ASMR2 model. The new model will also account for pond stratification, as in the Eighth Work Plan, Study 2 model. Initial model integration will be carried out in STELLA™, the simulation modeling language used for development of the Eighth Work Plan models.

2) *To program the model in a form that is "user-friendly" and can be run without specialized programming knowledge, or software packages.* The OSU Biosystems Analysis Group has been very successful with their development of POND©. They now have had over 900 downloads of the program from their Internet web site (J. Bolte, personal communication, September 1997). Their success is due in large measure to their efforts at making their work accessible by creating user-friendly interfaces for their models. We will work with the OSU Biosystems Analysis Group in developing the architecture for the new model coding, and in endeavoring to make the user interfaces consistent with the POND© interfaces. The annual meeting with the OSU Biosystems Analysis Group will be used to explore issues related to model architecture, to determine the extent to which some of the code developed for POND© (for actions such as data input and output, file creation and management, etc.) can be used in the new model, and to maintain consistency in user interfaces to take advantage of the POND© user base.

Regional Integration

The proposed research is "cross-cutting" and will apply to all the regions in which the PD/A CRSP has been active. Data used for model development, calibration, and validation will be from the PD/A CRSP Database. The model will be tested with data from all the regions.

Relative Contribution to Geographical Regions

The models developed will be most useful in regions for which data are available for model calibration and validation. Given the extent of the PD/A CRSP involvement, and the magnitude and scope of the Data Base for the various regions, the relative contribution is estimated as: 30% SE Asia, 30% Africa, 30% Central America, and 10% South America.

Schedule

Integration of the models will take place during the first year, and a complete, calibrated version will be available by April 2000. This initial version will be developed in STELLA™. A parallel effort at programming the models in C++ will be started in May 1999. By April 2000 a preliminary version of the integrated model and a user's manual will be available for distribution and testing. Comments and suggestions received from users will be incorporated in a version for general distribution by April 2001.

Report Submission

Progress reports will be submitted annually. A user's manual for the model will be available for distribution with a preliminary version of the software by April 2000. A revised version of the software and of the manual will be available for distribution by April 2001.

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Effluents and Pollution

Introduction

Effluents and Pollution research falls within the Environmental Effects research area outlined in the PD/A CRSP Continuation Plan 1996-2001. Research under the Ninth Work Plan addresses the goal of minimizing the detrimental environmental effects of aquaculture operations.

Feeding 17α-methyltestosterone (MT) to developing tilapia fry is an effective means of producing monosex populations; nevertheless, alternative methods require investigation because of the concerns raised about production of steroid wastes and metabolites that are potential environmental contaminants. Results from one Eighth Work Plan study showed that feeding tilapia with MT-treated food resulted in considerable "leakage" of MT into the water and soil of model ponds. Although the MT in the water disappeared shortly after withdrawal of the fish from the MT food, MT remained detectable in the soil for up to three weeks past this time. In the Ninth Work Plan, proposed studies will examine if there is long-term persistence of MT in the soil of "model" ponds, if MT is detectable in soil from ponds at CRSP sites, and if MT-contamination of soil has any effect on sex differentiation in tilapia.

Fate of Methyltestosterone in the Pond Environment

Effluents and Pollution Research 2 (9ER2)/Experiment, Study, Experiment

Collaborating Institution
Oregon State University
Martin Fitzpatrick
Carl Schreck

Objectives

1) To determine the duration of 17α-methyltestosterone persistence in pond sediment after dietary treatment of tilapia with 17α-methyltestosterone.
2) To determine if pond soil from CRSP site(s) contain detectable amounts of 17α-methyltestosterone.
3) To determine if sex differentiation in tilapia can be affected by culture in systems that contain 17α-methyltestosterone-contaminated soil.

Significance

Treatment of tilapia fry with 17α-methyltestosterone (MT)-impregnated food for producing all-male populations is a common practice throughout the world. All-male populations offer the production advantage of enhanced growth potential (Green et al., 1997). However, significant "leakage" of MT into the pond environment may occur from uneaten or unmetabolized food. This leakage poses a risk of unintended exposure of hatchery workers as well as of fish or other non-target aquatic organisms. Furthermore, in some countries, pond sediments are dredged and used to prepare "soil" for crop production thereby spreading the risk of exposure to MT to terrestrial systems.

In the Eighth Work Plan, we conducted an experiment to examine the fate of MT in "model" ponds. Newly released (10 days post-fertilization) tilapia fry were stocked at 0.3 fish/cm^2 into 3.7-L jars that contained 3 L of water and 3 cm of soil. The fish were fed either control food or MT-impregnated food at 60 mg/kg food for 28 days. Water samples were collected prior to stocking the fish and at 7, 14, 21, 28, 35, 42, and 49 days after the onset of feeding; soil samples were collected prior to stocking the fish and at 28, 35, 42, and 49 days after the onset of feeding. Water and soil samples were analyzed for the concentration of MT by radioimmunoassay. Although the levels of MT in the water peaked at an average of 1.7 µg/L at 14 and 21 days after the onset of feeding, the concentration decreased to background level by 35 days after the onset of feeding (1 week after the end of treatment with MT-impregnated food). In contrast, the levels in the soil were 1.8 µg/kg at 28 days after the onset of feeding with MT-impregnated food and remained detectable in the soil at between 0.8 and 1.6 µg/kg through 49 days (3 weeks after ending treatment with MT-impregnated food). Thus, MT persists in the sediments for a considerable time after the end of treatment, posing a potential health risk to workers and an exposure risk to non-target fish and other organisms. Tilapia may disturb sediments when they build nests or search for food, leading to resuspension of MT from the soil into the water column. Thus, "rotating" the pond use from fry production to rearing or breeding will not reduce the risk of re-exposure.

The proposed work will examine if:

1) MT persists in the soil of "model" ponds for up to 3 months after cessation of treatment,
2) MT is detectable in soil from ponds at one (or more) CRSP sites, and
3) MT-contaminated soil affects sex differentiation in tilapia (i.e., are sediments acting as an MT reservoir?).

Anticipated Benefits

An assessment of MT persistence and fate in the soil will provide information on the potential risks of using MT-feeding technology to workers and to non-targeted organisms. Furthermore, determining when MT no longer can be detected in the pond environment will help to establish additional safety guidelines for the use of MT.

Collaborative Arrangements
The collection of soil samples for Study 2 will involve collaboration with personnel from the Africa Project.

Experimental Design
Analytical procedures for measuring MT were developed in the Eighth Work Plan and consist of a specific radioimmunoassay (RIA) for quantifying MT in soil.

Experiment A: Detection of MT in soil after treatment with MT food
Site: Oregon State University, Corvallis, OR

Laboratory Facilities: Oregon State University—Two aquaria containing a total of two males and six females for production of fry, 3.7-L chambers (containing 3 L of dechlorinated oxygenated water and 3 cm of clay substrate collected from Soap Creek fish ponds); 75-L tanks within a recirculating water system for growing fry after hormonal treatment; Waters 660E Multisolvent Delivery HPLC system for separation and detection (by ultraviolet light absorption) of MT. MT radioimmunoassay (RIA) for quantifying MT concentration.

Culture Period: 180 days for offspring.

Stocking Rate: 47 fry/container (corresponds to a stocking rate of 3,000 fry/m^2).

Water Management: Water temperature will be maintained at 28-30°C. Every week, one-half of the water will be exchanged in the 3.7-L containers for new aerated water; in the recirculating system, complete water exchange is estimated to occur every 120 min.

Other Inputs: none

Test Species: Nile tilapia (*Oreochromis niloticus*), Ivory Coast strain.

Sampling Plan: The experiment consists of two groups: 1) fry fed MT at 60 mg MT/kg food for 28 days; and 2) fry fed regular feed. Each group will be replicated. Water samples (25 ml) will be collected on Days 0, 28, 56, 84, and 112 from each 3.7-L container (prior to any exchange with new water); soil samples (3-5 g) will be collected on Days 0, 28, 56, 84, and 112; all samples will be extracted on Sep-Pak (water) or with ether (substrate). Samples will be analyzed for steroid metabolites of MT by HPLC and for MT concentration by RIA. The following water quality parameters will be measured weekly: pH, ammonia, nitrates, and temperature. Fish will be removed from the 3.7-L containers on Day 28 and placed in separate 75-L tanks within a recirculating system for the grow-out phase. At the end of the 6 month grow out period, the tilapia will be killed to determine if the treatment with MT resulted in masculinization.

Statistical Methods and Hypotheses: H_01: MT is non-detectable in water at any time during and 1 week after treatment of tilapia fry with MT-impregnated food; H_02: MT is non-detectable in substrate at any time during and 1 week after treatment of tilapia fry with MT-impregnated food. This is a descriptive study and therefore, statistical analysis is unnecessary for testing the null hypotheses (i.e., detection of any amount of MT in water or substrate will be sufficient for rejecting the null hypotheses). Sex ratios will be compared between MT-fed and control groups by Chi-squared test.

Deliverables: A technical report.

Schedule: Data collection, 10/98-3/99; technical report, 7/99.

Study B: Detection of MT in pond soil from a CRSP site
Site: Sagana Station, Kenya; and Oregon State University

Pond Facilities: Two 200-m^2 ponds that are being used in other studies for fry production (sex-reversed and untreated fry) will be used.

Culture Period: 180 days for offspring.

Stocking Rate: 3,000-5,000 fry/m^2 in each hapa.

Water Management: Static ponds.

Other Inputs: none.

Test Species: Nile tilapia (O*reochromis niloticus*).

Sampling Plan: The study consists of analysis of soil samples from pond(s) in normal use at the Sagana station. Sampling will be from a pond that is in use or has been used for sex inversion of tilapia fry. Soil samples (3–5 g) will be collected from beneath the hapas used for feeding fry MT-impregnated food and at 5, 10, and 15 m from the hapas. If possible, soil will be sampled on Days 0, 28, and 56 of feeding to encompass the conditions prior to, at the end of, and 4 weeks after treatment with MT. Soil samples will include the top 3 cm of soil within the ponds. All samples will dried at the Station and stored in whirl-paks before shipment to the US for analysis of MT. The samples will be extracted with ether and MT measured by radioimmunoassay (as described in Experiment A). At the end of the 6-month grow-out period, a subsample (n = minimum of 50) of tilapia will be examined to determine if the treatment with MT resulted in masculinization.

Statistical Methods and Hypotheses: $H_0 1$: MT is non-detectable in pond soil at any time or location within the pond during or after treatment of tilapia fry with MT-impregnated food. This is a descriptive study and therefore, statistical analysis is unnecessary for testing the null hypotheses (i.e., detection of any amount of MT in substrate will be sufficient for rejecting the null hypotheses). Sex ratios will be compared between MT-fed and control (or a theoretical 50:50 male:female population) groups by Chi-squared test.

Deliverables: A technical report.

Schedule: Data collection, 9/98–9/99; technical report, 12/99.
If possible, other CRSP sites will also be screened for the presence of MT in soil sediments.

<u>Experiment C: Impact of MT-contaminated soil on tilapia sex differentiation</u>
Site: Oregon State University, Corvallis, OR

Laboratory Facilities: Oregon State University—two aquaria containing a total of two males and six females for production of fry, 3.7-L chambers (containing 3 L of dechlorinated oxygenated water and 3 cm of clay substrate collected from Soap Creek fish ponds); Waters 660E Multisolvent Delivery HPLC system for separation and detection (by ultraviolet light absorption) of MT. MT radioimmunoassay (RIA) for quantifying MT concentration.

Culture Period: 365 days.

Stocking Rate: 47 fry/container (corresponds to a stocking rate of 3,000 fry/m^2).

Water Management: Water temperature will be maintained at 28–30°C. Every week, one-half of the water will be exchanged for new aerated water.

Other Inputs: none

Test Species: Nile tilapia (*Oreochromis niloticus*), Ivory Coast strain.

Sampling Plan: The experiment consists of two treatments: 1) fry cultured for 28 days in jars containing soil contaminated with MT; and 2) fry cultured for 28 days in jars containing untreated soil. To make the experiment realistic, contamination of the soil will be achieved by cycling 3 sets of fry through the common MT treatment (i.e. each cycle will consist of fry fed 60 mg MT/kg food for 28 days). Water from both treatments will be exchanged weekly without disturbing the soil. Each treatment will be triplicated. Soil samples (3-5 g) will be collected at the end of each cycle (i.e., every 28 days) and at the end of the 28-day exposure period, when the fry will be moved to 75-L tanks for the grow-out phase. At the end of the 6-month grow-out period, the tilapia will be killed to determine if the exposure to MT-contaminated soil resulted in masculinization.

Statistical Methods and Hypotheses: $H_0 1$: The sex ratios of fry cultured in MT-contaminated and in untreated soil will not differ. Sex ratios will be compared between MT-fed and control groups by Chi-squared test.

Deliverables: A technical report. The results of the investigations outlined in this work plan will be synthesized into recommendations concerning the use of 17α-methyltestosterone for masculinization of tilapia.

Schedule: Data collection, 10/99–3/00; technical report, 7/00.

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Marketing and Economic Analysis

Introduction

Three studies will be conducted in Ninth Work Plan in marketing and economic analyses related to the PD/A CRSP research efforts. The first project will study the development of Central American markets for tilapia produced in the region. Much of the PD/A CRSPs research efforts have resulted in increased yields and production of tilapia. If markets are expanded and developed, increased production will result in a decline of farm-gate prices that can have a negative impact on the incipient tilapia industry in Central America. This activity will identify potential new domestic markets for Central American tilapia that will avoid price declines from increased quantities supplied.

The second study in this area will develop estimates of social and economic returns attributable to PD/A CRSP technologies in Thailand. This study will extend work that was completed under the Eighth Work Plan that estimated economic and social returns to the CRSP research in shrimp production technologies in Honduras. Results of this study will be useful for the PD/A CRSP to justify continued funding by quantifying benefits and impacts of the research effort.

Finally, Rapid Economic Evaluation Tools will develop a technique that can assist researchers in screening possible technologies for pond trials. The technique will build upon previous work using cost-return analysis in a risk framework to judge farmer incentives to adopt new techniques even as they are still in the research process. It will be designed to be a practical tool for use by biological researchers, giving them an indication of future economic viability, as experimental designs are formulated, to increase the likelihood that their research has a high chance of future use.

Development of Central American Markets for Tilapia Produced in the Region

Marketing and Economic Analysis Research 3 (9MEAR3)/Activity

Collaborating Institution
University of Arkansas at Pine Bluff
Carole R. Engle

Objective
To conduct an analysis of potential Central American markets for tilapia and to develop marketing strategies for tilapia produced in the region.

Significance
Much of the PD/A CRSPs research efforts have resulted in increased yields and production of tilapia. If markets are not expanded and developed, increased production will result in a decline of farm-gate prices that can have a negative impact on the incipient tilapia industry in Central America. This activity will identify potential new domestic markets for Central American tilapia that will avoid price declines from increased quantities supplied.

A limited amount of work has been done on markets for finfish in Central America. The few studies that have been carried out focused on the catch from commercial fisheries in Panama (Matton, 1981) and in Costa Rica (Scheid and Sutinen, 1979). Head et al. (1994) developed market guidelines for saltwater cultured Florida red tilapia in Puerto Rico. Several studies conducted in the US have examined the potential to develop markets for tilapia (Crawford et al., 1978; Nelson et al., 1983; Galbreath and Barnes, 1981). More recently, Swanson (1995) described US market requirements for tilapia. Engle (1997a) interviewed intermediate seafood buyers in the US to determine the potential to increase sales of fresh and frozen tilapia fillets in the US. However, virtually no work has been done on the potential to develop domestic markets in Central America for tilapia. Engle (1997b) describes the domestic markets that have emerged in Columbia for Columbian and Ecuadoran-produced tilapia. Development of domestic markets diversifies market opportunities and functions to stabilize industries from external shocks common in export-oriented markets.

Anticipated Benefits
This activity will provide guidelines to develop domestic markets for tilapia in Central America and reduce market risk by developing more diverse marketing strategies. The Federacion de Agroexportadores de Honduras (FPX) currently sells to only one buyer in the US. More comprehensive market information will provide opportunities for Central American tilapia producers to take advantage of reduced transportation, storage, and handling costs by understanding volume, packing, and labeling requirements of the most important domestic market channels. This activity will provide information that will allow the tilapia industry in Central America to develop and access new markets, increase the volume of goods sold, diversify marketing strategies, and improve financial success of tilapia growers. Results of this activity will be published in English and in Spanish.

The primary direct beneficiary of this study will be the Central American tilapia industry. Tilapia growers will benefit from the market information made available to them and from the training on marketing strategies that will be provided through this grant. At the present time, they are struggling to develop and maintain export markets without the benefit of comprehensive market information and in competition with other tilapia exporting countries. This is particularly true for small-scale growers who have greater difficulty in meeting the volume and sizing requirements of export markets. The proposed training workshop will teach tilapia growers, small and large, how to develop and maintain markets so that they will be capable of adapting to changing market conditions. While the primary focus is on the Central American industry, information on how to develop a domestic tilapia market will be of interest also to Thailand, other Asian countries, and to African nations on how to develop domestic markets for new species or non-traditional species. The PD/A CRSP will be a secondary beneficiary because improvements in market development will result in industry expansion that will allow for greater adoption and need for PD/A CRSP research results. The overall impact of the PD/A CRSP will be greater as the industry grows in Central America. Honduran university students will also benefit from the experience and skills gained through participating in this project.

Activity Plan

Location: The activity will be conducted by UAPB economists and will target seafood markets in Honduras and in Nicaragua.

Methods: Market structure and performance will be evaluated for fresh and frozen fish products in Honduras and in Nicaragua. Market structure is influenced by the numbers of buyers and sellers, barriers to entry, product differentiation and vertical integration. The current market structure of the wholesale seafood sector in Honduras and in Nicaragua will be analyzed. Lists of wholesalers, distributors, and restaurant and supermarket buyers will be obtained through contacts with FPX, the Universidad de San Pedro Sula, the Instituto Tecnológico de Honduras, the Asociacion Nacional de Acuícultures de Honduras (ANDAH) in Honduras and with the Universidad Centroamericana in Nicaragua. A variety of potential market channels and levels will be evaluated. Faculty and students from these universities will participate in study design, interviews, data collation, and will review final analyses and reports. Survey instruments will be designed in Spanish to elicit descriptive information on distribution channels, constraints , and efficiencies, product differentiation, product forms, price structures, volume requirements, packaging, labeling, product form, price, and other perceived characteristics of fresh and frozen tilapia products and other finfish that are close substitutes for tilapia. Following pre-testing and revision, direct personal interviews will be conducted. Existing market channels for fish and seafood will be identified and characterized. Potential customers and characteristics of potential tilapia buyers will be determined and evaluated. Market trends will be documented. Potential target market segments will be identified.

Price information and trends will be used to estimate potential marketing margins for domestic markets in Honduras and in Nicaragua. Marketing costs, including transportation, processing, storage, packaging, and promotion will be estimated using standard techniques. Comparisons of marketing margins to marketing costs will provide estimates of the feasibility of developing domestic markets for tilapia. Based on the marketing information obtained, guidelines on specific market opportunities will be developed. These guidelines will specify volume, price, product form, size, and other requirements for each type of market outlet identified. A preliminary trip to Peru will serve to identify the extent of the need for market analyses in Peru and to collaborate with the PD/A CRSP investigators there.

Regional Integration

The Regional Plan for Latin America identifies several constraints to aquaculture development in the region that include: lack of adequate economic and marketing information, and poorly developed local markets. This activity will address these issues by developing a base of economic and marketing information, developing guidelines and recommendations for effective marketing strategies, and conducting workshops to disseminate the results. This activity supports the effort described in the Regional Plan to extend efforts into Northern Honduras by providing marketing support to the rapidly growing commercial tilapia industry there. Furthermore, this effort will enhance regional integration by including Nicaragua in the activity and to conduct a workshop in addition to the market analysis in Nicaragua as well as Honduras. The need for a similar effort in Peru will be evaluated over the course of this activity.

Schedule

Year 1 (1/1/99 - 12/30/99): Collect and analyze information in Honduras. Organize workshop in Honduras.

Year 2 (1/1/00 - 12/30/00): Publish results of Honduran activity in English and in Spanish. Collect, analyze, and publish market information on Nicaragua. Hold workshop in Nicaragua.

Report Submission

Year 1: Report on Honduran marketing results will be completed by 1/31/00.

Year 2: Report on Nicaraguan marketing results will be completed by 1/31/01.

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Economic and Social Returns to Technology and Investment in Thailand

Marketing and Economic Analysis Research 4 (9MEAR4)/Study

Collaborating Institutions

University of Arkansas at Pine Bluff
Carole R. Engle

Asian Institute of Technology
Harvey Demaine

Objective

To develop estimates of social and economic returns attributable to PD/A CRSP technologies in Thailand.

Significance

The Pond Dynamics/Aquaculture CRSP is a global research activity directed toward improving the reliability and efficiency of pond aquaculture production. The ultimate benefit of this effort will be the economic and social returns that represent the impact from farmers adopting new technologies developed by the PD/A CRSP.

Technical progress has been modeled as a lagged function of research expenditures (Chavas and Cox, 1992). This study identified and measured the length of time required to fully translate public research expenditures into economic benefits and estimated internal rates of return for research expenditures. In the Chavas and Cox model, there were no restrictions on substitution possibilities among inputs, joint estimation of the production technology, technical change, and the effects of research on technical progress using very disaggregate inputs. This approach required only a standard linear programming algorithm. Ayer and Schur (1972), Ardito-Barletta (1971) and others estimated social rates of return to the investment in public research.

White (1985), in a study valuing research as an intangible capital in agriculture using Tobin's *q* theory, estimated that the market value of public research capital to be 8.6 times higher than conventional assets. Private research capital was valued 5.2 times higher than conventional assets.

Fischer et al. (1996) used a random-effects within a Bayesian framework to analyze the effect of adoption of new wheat varieties in South Australia. Results showed that not all pieces of information added equally to knowledge about the innovation. It further showed that the acquisition of information was much slower than had been suggested by previous Bayesian models and could also explain laggards and partial adoption. Huang and Secton (1996) developed a general imperfect competition model to evaluate returns to a cost-reducing innovation. In an imperfectly competitive market structure, this study showed that farmers' incentives to adopt a mechanical harvester for tomatoes in Taiwan were attenuated because the benefits were reduced by oligopsony power of processors.

Dorfman (1996) used a multinomial probit model to model adoption decisions faced by farmers when there are multiple technologies that can be adopted in varying combinations. Results showed that, for adoption of possible sustainable production technology bundles, that the adoption decisions are significantly influenced by off-farm labor supply.

Fuglie (1995) developed a multimarket model to explore equity and efficiency implications of improving crop storage technologies. The rate of return to research on potato storage in Tunisia was estimated to be between 44% and 75%.

Anticipated Benefits

Results of this study will be useful for the PD/A CRSP to justify continued funding by quantifying benefits and impacts of the research effort. This study will provide the first estimates of the global social and economic returns generated by the PD/A CRSP. The results of this project will document the contribution that the PD/A CRSP research has made and will continue to make over time in both social and economic terms. This is essential to justify continued funding for the CRSP in the US and for host country support. This project will not produce a field tool, but will provide the CRSP with a measure of the returns to AID funds invested in research.

Activity Plan

Location: The activity will be conducted by UAPB economists, but will be based on data from Thailand. No facilities other than computers at UAPB are required. The host country will be involved in supporting the data collection, but the analysis will be done at UAPB.

Data to Be Used Include: Much of the data required are secondary data. These include costs of the research, size of the industry, acreage, prices, total area in production, total production in ha, and average production. Primary data that will be needed include information on the rates of adoption of the specified technologies. The authors have been in touch with a researcher at AIT who has expressed interest in participating in the project.

Methods: This study builds upon work in the Eighth Work Plan in which the theoretical model was constructed to estimate the economic and social returns to technology and investment in CRSP research in Honduras. In this study, the same theoretical model will be used to estimate returns to the research investment in Thailand. This study defines a point in time when CRSP-developed technology was introduced. A measurement point is then defined. The equations estimated use time series data in functions and not data for any one given pint in time. The analysis is done for a specific technology, not necessarily a specific country. If the adoption of a given technology crosses national boundaries, it can be included in the analysis and the overall impact will be higher. The model presented below has been reviewed by economists at Purdue University and at Texas A&M University. It is similar to another impact study we are doing in collaboration with John Sanders at Purdue on the InterCRSP work. Supply and demand equations will be estimated to identify consumer surplus and producer surplus. Research that leads to the development and adoption of new technologies reduces the cost of production which further causes a reduction in the price to the producers. The combined changes in the net gain to producers and the net gain to consumers is the social gain from research. This study hypothesizes that there is a net increase in the economic surplus resulting from development and adoption of technologies produced from CRSP-funded research.

Given the nature of the PD/A CRSP projects and in conjunction with the different groups involved in the PD/A CRSP projects, it is necessary to evaluate the net social welfare resulting from the implementation of these projects. Although welfare economics is concerned with policy recommendations, it can also be used as an evaluation tool to determine the social impact of a given project. In an attempt to measure the PD/A CRSP impact, a function describing the net social benefits can be estimated. While the different groups involved in these projects are usually not mutually exclusive and in conjunction with the compensation criterion, social welfare can be measured as follows:

$$w = \Pi_Q + CS_Q + PS_Q + E - G$$

where W is net social benefits (positive or negative); Π_Q is the profit or rent accruing to PD/A CRSP researchers; CS_Q is consumers surplus for the host country which can be measured as surplus for final consumers plus all forward rents; PS_X is producers surplus measured as rent inputs plus all backward rents plus surplus for raw materials; E is external benefits/costs; and G is the social overhead cost for PD/A CRSP programs.

Let $\Delta Q = Q_0' - Q_0$ denote the change in total quantity observed due to research, k denote the vertical movement factor of the supply curve. Social gain (SG) can be expressed as:

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$SG = kQ_0 \pm k\Delta Q$ where + is used for ex-ante and - for ex-post evaluations.
Q is known; k and ΔQ are unknown and need to be estimated.

The necessary parameters to be estimated include the following: increase in productivity (ΔR) in kg/ha; adoption cost (ΔC) in terms of acreage moved from one activity to a new activity; adoption rate (t) in terms of % increase in acreage devoted to the activity (or in terms of new entrants); total area in production (S) in ha; total production (Q) in metric tons; average production/productivity ($R = Q/S$) in same as Q. The following will be estimated:

1. Let $J = \Delta R * t * S$. J can be viewed as the total increase in production due to technology adoption, holding cost and prices constant. Let j be the change in supply or coefficient by which the supply curve has moved with the new technology, $j = (\Delta R * t)/R = J/Q$.

2. $I = \Delta C * t/R$. I is the increase in cost of inputs per unit necessary to achieve J. I can be calculated proportionally to observed price (P) such that $c = I/P = (\Delta C * t)/(R * P)$.

3. Let $K = (b * J) - I$, where b is the supply curve slope; K represents the net reduction in production cost due to technology (vertical movement of the supply curve). In fact, the coefficient b is not used, the supply elasticity (ε_S) is used instead.

$\varepsilon_S = \Delta Q/\Delta P)*(P/Q) = (1/b)*(P/Q)$. This leads to $\varepsilon_S*b = P/Q$, and $b = (1/\varepsilon_S) * (P/Q)$; therefore,
$K = [(1/\varepsilon_S) * (P/Q) * J] - I = (P * j/\varepsilon_S *Q) - I$. With respect to price (P),
$k = K/P = [P * J/\varepsilon_S * Q) - I]/P = (P * J/\varepsilon_S * Q * P) - (I/P) = [(1/\varepsilon_S) * (P * J/Q * P)] - (I/P)$
$k = (1/\varepsilon_S) * j - c$
when supply is inelastic ($\varepsilon_S < 1$), an increase in production due to research has a relatively high economic value ($k > j - c$), possibly limited acreage. Elastic supply ($\varepsilon_S > 1$), possibly abundant acreage, ε_S reduces k ($k < j - c$). In this latter case, it is easy to increase production and research gains have little economic value.

Regional Integration

The Regional Plan for Asia includes the need to monitor the impact of the CRSP and its returns to both the research community and the local aquaculturists.

Schedule

Year 1 (5/1/99-4/30/00): Collect and analyze data from Thailand necessary to estimate the above-mentioned model.
Year 2 (5/1/00-4/30/01): Estimate the above-mentioned model with the data collected and prepare a manuscript for publication.

Report Submission

Year 1: Annual report will describe the data collection efforts.
Year 2: The final report will be completed by 4/30/01.

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Rapid Economic Evaluation Tools*

Marketing and Economic Analysis Research 5 (9MEAR5)/Study

Collaborating Institution(s)

Auburn University
Upton Hatch
Co-PI (TBA)

Honduras US PI (TBA)

Objectives

1) To evaluate alternative aquaculture production systems (i.e., the different established PD/A CRSP pond nutrient input strategies) in terms of both profitability and risk.
2) To develop a capability to fine-tune technologies based on cooperation with farmers.

Techniques will be developed to evaluate the profitability and riskiness of aquacultural systems. These techniques will be packaged into "user-friendly tools" that CRSP biologists and others can use to quickly establish if a tilapia production strategy is likely to be profitable and easily implemented by producers, and at what level of risk to the grower. This preliminary profitability and risk determination procedure will allow CRSP researchers to use their limited resources more efficiently by concentrating their research on production strategies deemed most likely to be profitable at an acceptable risk level.

Significance

The goal of CRSP is to increase the availability of animal protein by developing appropriate pond aquaculture systems. The adoption of the developed pond aquaculture systems depends in great part, among other things, on the expected net returns. Aquaculture as a farm enterprise is not without its risks. The proposed economic research differs from many economic evaluation processes in that it explicitly addresses economic risk, will be developed for use by interdisciplinary researchers, and focuses on developing research treatments that are likely to be implemented by farmers.

The goal will be a technique that can assist pond researchers in screening possible technologies for pond trials. Economic indicators will be used to allow researchers to put priority on treatments that pass an initial economic screening. Thus, economic criteria will be integrated with biological ones in targeting research to the most promising technologies for the end users—the farmers. The technique will be "preliminary" in the sense that it will be a first screening of potential technologies before they have been used extensively in the field and will be based almost exclusively on research pond results. A more in-depth economic evaluation would follow several years later, based on on-farm results and detailed analysis of the adoption process (not in the scope of this proposal).

Anticipated Benefits

When the technique is successfully developed using data from CRSP countries, it can be disseminated to other sites and will be put in practice, where possible. The goal for this proposed research will be a technique that will be easily implemented by biological researchers with a minimum of consultation with an economist.

Identification of Beneficiaries

Farmers need improved techniques in producing protein for family nutrition. The proposed techniques should allow a more focused research effort on tilapia growing techniques that are likely to be adopted by farmers, be they subsistence-, small-, or commercial-scale farmers.

Although the proposed effort will focus on tilapia in Honduras in 1998–2000, the technique should be rather easy to adapt to other locations and species. In general, it might be expected that the technique would be easier to apply to tilapia production in other areas than to other species. Since it is envisioned

* As of the time of printing, the PMO has approved this investigation for funding, but the formal subcontract for work is still in process.

to be dependent almost solely on pond research data, differences in local growing conditions for a specific species would be reflected in the pond results. The technique's applicability to other species may be less clear, since some species may have important economic differences, for example, price differences by size and dependence on inputs not readily available in the local area.

Both the economic results and the technique could make an important contribution to the Data Analysis and Synthesis Team (DAST) expert systems aquaculture model. The technique proposed might be useful to the DAST effort by providing an early indication of economic feasibility for a given tilapia technology toward a specific target population of farmers, i.e. subsistence-, small-, or commercial-scale farmers.

Collaborative Arrangements

The International Center for Aquaculture and Aquatic Environments (ICAAE) has made a major commitment to interdisciplinary research and extension. The results of this commitment have been the development of a cadre of expertise in the sciences and social sciences focused on the development of economically viable and environmentally sound aquaculture production systems. Collaboration on this project will be with the principal investigator at the Honduras site and with the CRSP/DAST team. Most collaboration will be in the form of interviews to understand the CRSP experimental data or for data retrieval from the CRSP aquacultural database.

Experimental Design/Methods

Baseline fish production data (nutrient inputs, stocking rates, etc.) collected during the course of CRSP experiments over the last ten years will be used. In addition, prices of nutrient inputs and sales prices of fish and any seasonal fluctuations will be obtained from CRSP country PIs. Using these data, economic indicators of profitability and risk will be determined. Expected enterprise returns are not known with certainty, but have an associated distribution of returns. A large expected profit may have associated with it a wide distribution, and thereby a higher economic risk. Inherent seasonal price distributions for inputs and outputs will be incorporated into the analysis by using available spreadsheet software.

Site(s): Sites involved include Honduras. Project staff will perform most of the work at Auburn University, but will work closely with Honduras project staff. One trip to Honduras will be required each year.

Research Plan

The plan is to concentrate on the fish production, pricing, and environmental data from Honduras during the first year while the software is being programmed and refined.

Identification of Deliverables

1) An evaluation of alternative aquaculture production systems (i.e., the different established PD/A CRSP pond nutrient input strategies) in terms of both profitability and risk.
2) Techniques will be developed to evaluate the profitability and riskiness of tilapia production systems in general.
3) These techniques will be packaged into "user-friendly tools" for use by biologists in the field and also provided to the DAST element of the PD/A CRSP for incorporation into their financial analysis program.

Schedule

During 1999 and 2000 the focus will be on development of the profitability and risk determination algorithms. Data from the Honduras CRSP freshwater tilapia experiments will be used initially. As the programs are tested and refined, interviews with local farmers will be used to fine tune the analysis. The method could be tested at additional CRSP sites (not included in this proposal) to further expand the robustness of these analytical tools.

Final Report Submittal

A final report will be submitted by 30 April 2001.

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Decision Support Systems

Introduction

Aquaculture managers are confronted with complex decisions for culture facilities, effects on the environment, and the role in larger farming systems. Analytical tools for decision support systems integrate knowledge as mathematical models, expert systems, and databases into software systems. The PD/A CRSP has developed POND©, a software package that provides capabilities for simulation modeling and economic analyses of pond facilities. POND© and similar decision support systems are potentially valuable tools for assessing the economic and ecological impacts of alternative decisions on production, and for increasing understanding of the interrelationships which can control production dynamics. By capturing the fundamental principles affecting pond production, coupling these with appropriate economic analyses, and presenting results in a readily understandable manner, these decision support tools can improve the design, management, and analysis of production facilities.

Within the Ninth Work Plan, the PD/A CRSP will continue to improve and refine aquaculture decision support tools, focusing on 1) improving the utility of POND© software for education and extension purposes, 2) enhancing POND©s underlying models in the areas of pond sediment-water column interactions, 3) calibrating and validating POND© for additional culture organisms, particularly various shrimp species, and 4) enhancing POND©s ability to address scheduling and other applied pond management issues. These improvements should improve the usefulness of the software in addressing the needs of both educators and pond managers, and allow improved decisionmaking in areas related to fertilization, feeding, stocking, water use and effluent discharge, and economic optimization.

Decision Support Systems for Fish Population Management and Scheduling in Commercial Pond Aquaculture Operations

Decision Support Systems Research 2 (9DSSR2)/Study

Collaborating Institutions
Oregon State University
John Bolte
Marion McNamara

University of Arkansas at Pine Bluff
Steve Killian

Cooperating Institution
University of Georgia
Shree Nath

Objectives
1) To modify previously developed fish growth models so as to enable simulation of population growth of multiple fish lots in pond environments.
2) To develop methods for estimating fish biomass in ponds that are stocked and harvested at various time intervals in continuous production systems.
3) To implement software support for inventory management of fish stocks in operational farms.
4) To provide training to farmers on the use of decision support software for routine pond management.

Significance
Commercial, large-scale pond aquaculture operations (e.g., catfish, tilapia, and shrimp) are increasingly dependent on continually supplying fish to markets or to processors. Common to these operations is the practice of multiple stocking and harvesting, which results in several fish lots in a pond at any given time. Each lot tends to grow as a distribution (in terms of fish weights) because of genetic factors, sex differences, food competition, and behavioral changes. Distribution profiles of individual lots are also modified as a result of selective harvest practices (e.g., grading and sorting, and high-grade harvesting). Our previous growth modeling efforts (e.g., Nath, 1996; Nath and Bolte, 1998) assumed that all fish in a particular lot grew uniformly. Consequently, the POND© software currently simulates mean fish weight for a given lot over time. The proposed study will involve refinement of POND© models to enable prediction of fish weight distributions over time. This effort will draw upon current PD/A CRSP research at the University of California at Davis as well as previous work on modeling fish and shrimp distributions in ponds (e.g., Griffin et al., 1981; Cuenco et al., 1985; Leung and Shang, 1989). Methods will also be developed to estimate total fish biomass of multiple lots in ponds at different time intervals. Parameterization of the population growth models will be accomplished for tilapia (using CRSP data) and for channel catfish (using data from growth experiments and on-farm trials in the Southern US). Similar parameterization for shrimp will be undertaken in the future.

Users of the POND© software have requested support for inventory management of fish lots. It is anticipated that addition of the above functionality within a decision support framework will be valuable to farmers in the context of accurately estimating fish biomass, scheduling pond harvests, and assessing feed conversion efficiency. Modifications to POND© are also expected to be useful to extension agents and managers interested in projecting farm performance and assessing production risks.

Another important component of the proposed work is the training activity, which will involve a workshop to familiarize US farmers with application of pond management software developed by the PD/A CRSP. The workshop will be organized by the Education Development Component of the CRSP.

The proposed study falls primarily within the area of **Social and Economic Aspects** identified in the PD/A CRSP Continuation Plan 1996-2001. Specifically, the work is relevant to the *Decision Support Systems* research theme, and should generate recommendations that would assist farmers both overseas and in the US to optimize scheduling operations and fish supply to markets.

Anticipated Benefits

The proposed study is expected to result in the following benefits:

1) Models for projecting fish weight distributions over time, and estimating harvest events
2) Improved capabilities for managing multiple fish lots and their distributions in ponds
3) Support for inventory management of ponds and lots in existing facilities
4) Improved support for economic analysis of commercial pond operations
5) Farmers trained to apply decision support software for real-time management

Research Design

Activities proposed in this study include the following:

1) *Models for Size Distribution Analysis*: This activity will primarily involve refinements of the POND© bioenergetics model to enable analysis of fish weight distributions in ponds. Refinements will include definition of size classes (bins) within statistical distributions, and capabilities of simulating differential fish growth (within and among bins) for individual lots between stocking and harvest intervals. Differential growth will be modeled as a function of both intrinsic factors (e.g., competition for food resources) and extrinsic factors (e.g., harvest events).
2) *Decision Support for Scheduling Harvest Operations and Assessing Economic Outcomes*: This activity will involve development of user interfaces and decision pathways to enable farmers to prescribe multiple stocking/harvest schedules for ponds, and to be able to interactively run various scenarios that result in economically optimal production. Economic analysis will be accomplished by linking the present enterprise budgeting functionality to the management scenarios prescribed by the user.
3) *Inventory Management*: This activity will involve considerable refinement of the databases in POND© to enable farmers to enter and edit multiple stocking/harvest, feeding rate, and feed conversion data relevant to fish lots in ponds located at their facility. Capabilities will also be developed to enable farmers to modify these data in other types of software (e.g., spreadsheets) that they use on a routine basis. Finally, support will provided for farmers to manually modify predicted statistical distributions of fish weights based on harvest data.
4) *Farmer Training*: This activity will involve the development of training materials for a workshop on the use of software developed for size distribution and inventory management, coordination of the workshop (to be conducted at the University of Arkansas at Pine Bluff), and evaluation of software and training materials by farmers.

Regional Integration

Modifications to the POND© software should be of benefit to users in all the CRSP sites. In terms of the level of effort among the four CRSP regions, it is anticipated that the study will be distributed evenly.

Schedule

Timelines for the proposed study and lead institutions for various activities are summarized in Table 1.

Report Submission

Quarterly progress reports and an annual report will be submitted consistent with the PD/A CRSP timelines for report submission.

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Table 1. Timelines for project activities (OSU = Oregon State University; UAPB = University of Arkansas at Pine Bluff).

Activity	**Institutions**	**Timeline**
Models for Size Distribution Analysis	Lead: OSU Support: UAPB	July 1, 1998 to June 30, 1999
Decision Support for Scheduling Harvest Operations and Assessing Economic Outcomes	Lead: OSU Support: UAPB	January 1, 1999 to December 31, 1999
Inventory Management	Lead: OSU	July 1, 1999 to June 30, 2000
Farmer Training	Lead: OSU	May 2000

Enhancing the POND© Decision Support System for Economics, Education, and Extension

Decision Support Systems Research 3 (9DSSR3)/Study

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Cooperating Institutions
University of Michigan
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Central Luzon State University, Philippines
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Wageningen University, The Netherlands
Johan Verreth

Oregon State University
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Objectives

1) To enhance the POND© decision support system's ability to conduct economic analyses for commercial aquaculture facilities.
2) To modify and calibrate POND©s bioenergetic models to allow the simulation of growth, development, mortality, and yield of shrimp species in culture ponds.
3) To incorporate feed optimization capabilities into POND©.
4) To develop training and education materials to support and enhance the use of POND© in extension and academic settings.
5) To improve the user interface of POND© to support analyses of common facility management tasks.

Significance

The POND© software (Bolte et al., 1995; Nath et al., 1997; Nath, 1996) is a robust and powerful decision support tool developed by the Pond Dynamics/Aquaculture Collaborative Research Support Program (PD/A CRSP) to assist in the analyses of fish growth and pond dynamics in warmwater culture situations. POND© incorporates a multilevel bioenergetic model of culture organism growth and development that has been successfully calibrated for a number of fish species (Nath, 1996), as well as a simple enterprise budgeter capable of basic cost/income accounting. POND© provides estimates of feed and fertilizer requirements to satisfy growth requirements, or alternately allows simulating the effect of specific feed and/or fertilizer regimes on fish production and water quality. Further, POND© allows for very flexible water budget estimation and can simulate water temperature profiles based on local climatic conditions and pond physical characteristics. The various submodels in POND© have been widely validated and tested, and have generally produced good prediction of pond and facility dynamics over a wide range of facility configurations and geographic conditions.

The POND© software has been available to end users via the World Wide Web for the past eighteen months. In that period of time, over 900 users have downloaded the software. As the user base of POND© has grown, the need for a number of modifications and enhancements to the current POND© capabilities has become apparent. The work proposed here seeks to incorporate user feedback to make POND© more useful for addressing demand for enhanced economic analyses of pond facilities, optimized feed scheduling, using POND© in a classroom environment, calibrating POND© for shrimp species, and improving POND© user interface.

The primary significance of the proposed work is in enhancing POND© utility as a tool for more effectively managing commercial production facilities. The models incorporated into POND© have demonstrated themselves to be useful tools for synthesizing the results of the PD/A CRSPs and others' experimental results into a framework capturing the many interactions and interrelationships present in aquacultural production systems. The primary need for additional work now is to bring the previous efforts on developing core models of fundamental facility processes to bear on applied management.

The proposed study falls primarily within the area of **Social and Economic Aspects** identified in the PD/A CRSP Continuation Plan 1996-2001. Specifically, the work is relevant to the *Decision Support Systems* research theme, and should result in improved software tools for commercial aquaculture producers to plan and manage their facilities, and for instructors involved in academic aquaculture programs.

Anticipated Benefits

The proposed study is expected to result in the following benefits:

1) Enhanced ability to perform sophisticated economic analyses of production facilities.
2) Enable the analysis and management of pond-based shrimp culture facilities.
3) Development of feed optimization strategies for pond management.
4) Improved student understanding of pond dynamics and production management principles.
5) Enhanced capability to utilize POND© in applied pond management and facility planning.

Research Design

Feedback from POND© users has revealed several strong trends in user requirements for future development of POND©. The feedback has come from the comments from the POND© user base and serves as the driving factor for the work proposed here.

First and foremost, a requirement for doing more sophisticated economic analyses with POND© has been cited by approximately one-half of those providing feedback. The current POND© implementation is limited to simple enterprise budgeting, with the ability to incorporate simulation results that only reflect fish production. No feed, fertilizer or other resource costs resulting from the simulation are utilized directly in the enterprise budget; these items have to be explicitly entered, and are not synchronized with the facility simulation results. Further, the ability to associate time frames with cost/income items, or to schedule costs or harvest income is not currently supported. The analysis options associated with the resulting enterprise budget are limited, showing only a basic net income, rather than more sophisticated and useful measures of economic performance such as return on investment (ROI), marginal cost vs. return and partial budgets, etc. Because of the high demand for more sophisticated economic analyses in POND©, we propose to address the deficiencies described with a more robust economic analysis package providing time-based and scheduled costs, tighter integration with facility simulations, and a wider range of reporting statistics.

A second area generating a high level of requests for enhancement of POND© involves the inclusion of models of shrimp species growth and development. Because the current bioenergetic model in POND© is fairly mechanistic and relatively species-independent, we have some expectation that we will be able to develop calibration parameters that provide good predictive capabilities of production dynamics. However, because of fundamental differences between the developmental cycles of shrimp and most cultured fish species, some modification to the basic bioenergetic model may be required, particularly to adequately estimate development during the early postlarval stages. We propose to calibrate and enhance the bioenergetic model to allow POND©-based analyses of shrimp culture facilities.

As high-intensity fed systems become more important in commercial aquaculture, the ability to account for the effects of feed quality on production and to optimize feed utilization and expense becomes important. Feed input is frequently the highest variable cost associated with production. The bioenergetic model in POND© is well-suited to account for feed quality and quantity effects of production dynamics, and POND© currently includes these effects. We propose to refine the consideration of feed quality and expand the current linear programming-based fertilizer optimizer to encompass feed optimizations as well. Both formulation and optimizing feed quantity scheduling through the production will be incorporated into POND© . This will greatly enhance the utility of POND© for examining feed-driven production facilities from both biological and economic perspectives.

A fourth area for which the POND© user community has expressed a high degree of interest is the use of POND© in a classroom environment. Because POND© synthesizes many aspects of pond dynamics and facility management, it has the potential to be used in a classroom setting to allow students to gain an understanding of the interrelationships between various aspects of management, production, and water quality through what-if scenario development. POND© has been tested in an aquaculture management course at the University of Michigan (J. Diana, personal conversation) with mixed results. The main difficulties expressed by students using the software dealt with complexity of the interface and the lack of tutorials relating the use of POND© to address specific management questions. We propose in this workplan to work with four listed cooperators to:

1) Develop enhanced user interfaces for assisting students and producers to use POND© to address common, specific management tasks;
2) Develop instructional materials to use in conjunction with POND© to enhance its utility as an educational vehicle; and
3) Evaluate the effectiveness of these enhancements for developing student understanding of pond dynamics.

Finally, we have recently explored the use of "wizards" to facilitate common tasks in POND© (Nath and Bolte, 1998). The wizard approach has shown substantial potential to simplify the user interface and move POND© from an analysis tool primarily for research to a more practical tool for applied commercial pond management and facility planning. We propose to further develop this concept and focus effort on interface improvements to make the POND© models more accessible for applied users, and more focused on solving coming management tasks.

Specific research activities will therefore focus on the following:

1) *Enhanced Economic Analyses and Enterprise Budgeting*: POND© currently contains a software "economist" that is capable of basic enterprise budgeting. This economist will be expanded in its ability to associate time periods with its various cost and income items, and to allow scheduling of costs to reflect seasonal or production cycle-related activities. This will allow the economist to track the dynamics of profitability and allow the determination of optimal harvest scheduling based on marginal cost/marginal return analyses. Additional economic indicators, including ROI, NPV, etc., will be incorporated into the economist. Partial budgets will similarly be supported. The enterprise budget reports will be modified to be consistent with the other PD/A CRSP enterprise budgeting activities. The economist user interface will be simplified and improved through the development of two additional wizards, to guide the user in defining production costs, and to facilitate enterprise budget analysis.
2) *Calibration of POND© Bioenergetic Model for Shrimp Species*: We will attempt to calibrate the bioenergetic model using existing shrimp growth datasets using POND© automatic calibration facilities. These datasets will be extracted from the PD/A CRSP Database and additional data sources. If modifications to the bioenergetic model are needed, these will be made based on other shrimp growth models (e.g., Leung and Shang, 1989).
3) *Incorporating Feed Optimization into POND©*: POND© currently contains a general-purpose linear programming (LP) optimizer applicable to any numerical optimization problem involving linear constraints. We currently use this optimizer to develop optimal (least-cost) fertilizer formulations to meet specific overall fertilizer demands. This optimizer will be adapted to perform similar functionality for feed formulation. Additionally, POND© contains a genetic algorithm-based nonlinear combinatorial optimizer currently used for parameter estimation. This optimizer will be adapted for use in generating optimal feed schedules to meet production weight targets for particular harvest schedules, based on minimizing overall feed costs.

4) *Training and Educational Material Development*: A series of modules addressing the use of POND© in applied planning and management will be developed in conjunction with the listed cooperators. The specific coverage of the modules will be determined in consultation with the cooperators, but is expected to be 1) basic familiarity with the use of POND©, 2) facility capacity planning and production analysis, 3) production optimization, 4) fertilization rate determination, 5) feed rate determination, 6) water budgeting, 7) basic economic analyses, and 8) advanced economic analysis. We will monitor the use of these modules in a classroom setting and survey students as to the effectiveness of the tutorials in improving their understanding of aquaculture management.
5) *Improving the POND© User Interface*: Many of the tasks listed above will require improvements in the POND© user interface. We will replace most task-oriented components of POND© with intelligent "wizards" capable of walking users through a specific task, explaining options associated with each task, and assisting in interpreting the results. Other incremental improvements taking advantage of advances in the underlying Win 95/NT operating system will be incorporated in POND© as well.

Regional Integration

Modifications to the POND© software should be of benefit to users in all the CRSP sites. In terms of the level of effort among the four CRSP regions, it is anticipated that the study will be distributed evenly.

Schedule

Activity	*1998*		*1999*				*2000*	
	Q3	*Q4*	*Q1*	*Q2*	*Q3*	*Q4*	*Q1*	*Q2*
Economic Analysis Enhancements	XX	XX	XX	XX	XX	XX		
Feed Optimization	XX	XX						
Shrimp Calibration and Validation			XX	XX	XX	XX		
Training Material Development and Evaluation			XX	XX	XX	XX	XX	XX
User Interface improvements	XX	XX	XX	XX	XX	XX	XX	XX

Report Submission

Quarterly progress reports and an annual report will be submitted consistent with the PD/A CRSP timelines for report submission.

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REGIONAL RESEARCH

The CRSP Ninth Work Plan encompasses research, training, and extension activities to be carried out in three major regions of the world—South America, Africa, and Southeast Asia. The South America project is located in Peru, the Africa project operates out of Kenya, and the projects in Southeast Asia are composed of an island/oceanic component in the Philippines and a mainland component in Thailand. In addition, under a restricted request for proposals issued by the Management Entity in March 1999, a Central America project sited in Honduras will likely be in place by mid-1999.

The prime site for South America was established under the Eighth Work Plan at Quistacocha Research Station, Iquitos, Peru, in the Loreto Region of the Peruvian Amazon, where the CRSP collaborates with the Instituto de Investigaciones de la Amazonia Peruana and the Universidad Nacional de la Amazonia Peruana. Under the Ninth Work Plan, the site will be further developed, including enhancement of the water quality lab and pond and hatchery facilities. The objectives of specific research activities will continue to be the development of alternative species for culture, with a focus on broodstock maintenance, including nutrition, induced spawning using hormones, and analysis of seasonal plasma steroid levels. Pond grow-out studies will also be performed to build upon results of pond studies conducted in the Eighth Work Plan. Training of Peruvian personnel will continue, focusing on water quality analyses, the use of hormones as spawning aids, and proper nutrition of broodstock. Outreach and networking activities will be undertaken to regionalize the benefits of the CRSP.

The Africa project operates out of Sagana Fish Farm, Sagana, Kenya, in collaboration with the Kenya Fisheries Department. Plans also include the establishment of at least one companion site in the East Africa region where relevant aquaculture research can be conducted. Research to be conducted at Sagana under the Ninth Work Plan includes a feed study to determine the best combination of locally available feedstuffs for use in farmers' ponds and a study on the use of pond effluents in crop production on adjacent field plots. Other activities to be carried out under the Ninth Work Plan include aquaculture training for fisheries extension officers and students at both graduate and undergraduate levels, on-farm trials of stocking and management options, and outreach efforts to establish and encourage linkages and collaboration among regional aquaculture institutions, including the CRSP.

The Philippines project is based at Central Luzon State University (CLSU), but may also include research on other islands and in other areas of the Philippines. Project coordination and integration will be improved by strengthening electronic networking, allowing CLSU to function effectively as a regional prime site in support of aquaculture throughout Southeast Asia. Ninth Work Plan activities include on-farm trials of timing of the onset of supplemental feeding and reduction of rations below satiation levels, as well as workshops and production of improved extension materials. The Thailand project is based at the Asian Institute of Technology (AIT), near Bangkok, but has in recent years included outreach activities out of Udorn and other fisheries stations in the region. Under the Ninth Work Plan, research will also be conducted at sites in Vietnam and Laos and opportunities for work in Bangladesh, Sri Lanka, and Nepal will be explored. It is hoped that a high-elevation site, which might provide comparable information to the African site, can be developed. Studies undertaken out of AIT will include studies on polyculture of mixed-sex tilapia with snakehead, integrated recycle systems for catfish and tilapia, and taro culture in fish ponds to recycle pond mud nutrients.

Peru Research

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Spawning and Grow-Out of *Colossoma macropomum* and/or *Piaractus brachypomus*

New Aquaculture Systems/New Species Research 3
(9NS3)/Study, experiment, study, experiment, study

Objectives

1) Identify locally available ingredients for practical diet(s) suitable for *Colossoma* and/or *Piaractus* broodstock.
2) Determine blood plasma steroid concentrations of *Colossoma* and/or *Piaractus* broodstock during the annual cycle and preceding ovulation/spermiation in relation to gamete quality.
3) Compare use of hCG and GnRH for inducing *Colossoma* and/or *Piaractus* to spawn.
4) Identify proper density for pond culture of *Colossoma* and/or *Piaractus.*
5) Determine cost of production for rearing *Colossoma* and/or *Piaractus* in ponds at different densities.

Significance

A need exists to evaluate the aquaculture potential of local and native species, and to develop appropriate culture technologies. *Colossoma* spp., *Piaractus* spp., and their hybrids are important food fishes in the Amazon basin. However, little production technology has to date been developed and published. In addition, there has been inadequate attention to economic analyses, such as determinations of cost of production.

Male and female *C. macropomum* generally reach sexual maturity in 3 and 4 years, respectively, when they have attained 3 to 6 kg in weight. *Piaractus* become sexually mature about a year sooner, and at a smaller size (2 to 4 kg). Fish held in captivity must be induced to spawn using hormones (Gonzales, 1987). No standardization in terms of hormone type or dosage exists. After hormonal treatment gametes are stripped and mixed using the dry method (Alcantara and Guerra, 1992). Both MacDonald-type and Woynarovich-type incubators are employed, with the latter being most common. Hatching occurs 10 to 20 h post-fertilization at 26 to 29°C. In 1996, a complete spawning failure occurred at the IIAP station in Iquitos, as well as at another station in Peru (Tarapota). The spawning failure may have been due to drought conditions at normal time of spawning. Other factors such as poor nutrition may have also been involved. Accordingly, we will monitor plasma steroid levels of broodstock on a monthly basis over the course of two years. These data will be related to climatological information, as well as the actual spawning events (or nonevents). These data will also be useful in identifying the optimal time for induced spawning. We will compare the relative efficacy of various dosages of hCG and GnRH to induce ovulation. We will also survey locally available feed ingredients and design one or more feeds for use in broodstock maintenance. Final maturation and ovulation was achieved in *Colossoma* sp. using hypophysation procedures and injections of commercial hormones (Carolsfeld, 1989). Information on reliable procedures of controlled gonad maturation leading to induced ovulation of captive fish is available for Brazil (Chellappa et al., 1996; Zaniboni Filho and Barbosa, 1996) and Venezuela (Gonzalez et al., 1991). The profile of steroid hormones in captive *Colossoma* sp. was recently analyzed by Gazola et al. (1996), however these data are incomplete. Steroid hormones analysis in conjunction with environmental conditions (water temperature and conductivity, light regime) and body condition factor (coelomic fat index; Lamas and Godinho, 1996) were not carried out systematically in tropical Characidae fishes. OSU preliminary data over the course of captive *C. macropomum* growth in North America suggest that the blood plasma steroid profiles are indicative for gonadosomatic indices of both sexes and can be critical in determining the time and magnitude of hormonal intervention. An insight into seasonal and final maturational-stage steroid profiles in males and females can contribute to an understanding of the process and synchronize induced ovulation with hormonal treatment. No standardization exists for stocking densities of fry or fingerlings (Campos, 1993). No uniform fish diets are available in the region (Cantelmo et al., 1986; Ferraz de Lima and Castagnolli, 1989). In the Eighth Work Plan, we stocked three ponds with 3,000 *Piaractus* fingerlings/ha and three at 4,000/ha. After approximately 120 days, the fish have grown from an average weight of 27.5 g to 353.6 g at the lower density and 417.2 at the higher density. The yield trial will continue for another 120 days. It appears that even higher densities may be possible, although water quality will become problematic at some point. In the Ninth Work Plan, research will be aimed at further refining stocking densities to efficiently and economically rear *Colossoma* and/or *Piaractus* to marketable size (= 1 kg).

Beneficiaries

The development of sustainable aquaculture of *Colossoma* and/or *Piaractus* will benefit many sectors throughout the Peruvian Amazon. Rural farmers will benefit by the addition of an alternative to other forms of agriculture. Aquaculture production will require considerably less land than that needed for cattle ranching. Moreover, ponds can be used year-after-year whereas rain forest lands converted to traditional agricultural practices are rarely productive for more than a couple of seasons, and such lands, once abandoned, usually can no longer support normal jungle growth. Both rural and urban poor will benefit by the addition of a steady supply of high quality protein in the marketplace. Aquaculture of *Colossoma* and/or *Piaractus* should relieve some of the fishing pressure on these overharvested, native species. These species have been shown to play a crucial ecological role in disseminating seeds from the flooded forest. Accordingly, the aquaculture of *Colossoma* and/or *Piaractus* will be ecologically as well as economically and nutritionally beneficial to the inhabitants of the Peruvian Amazon.

Collaborative Arrangements

SIUC will coordinate these activities. IIAP and UNAP will provide Host-Country PIs. OhSU and SIUC will participate in the plasma steroid study. UAPB and SIUC will participate in the broodstock nutrition study. SIUC will participate in the spawning, density, and cost of production studies.

Methods

Objective 1: The content of certain essential nutrients (e.g., amino and fatty acids) in fish eggs is quite conservative and nutrient profile of eggs of a given species is a good indicator of larval requirements. Because origin of the nutrients is primarily maternal, there is potential to manipulate broodstock diets to optimize oogenesis and larval quality. Accordingly, we will:

1) analyze the protein, amino acid, lipid, and fatty acid content of eggs of *Colossoma* and/or *Piaractus* (preferably from wild fish in good condition) using standard techniques (Kjeldahl, Folch, spectrophotometry, and chromatography);
2) assess availability and cost of practical feedstuffs in Peru; analyze nutritional components of those offering best potential for broodstock; and
3) formulate and manufacture one or two test diets; conduct trial (minimum of 10 broodfish for each treatment) to compare influence on reproductive performance (see objective 3) of test

diet(s) to diet currently being fed (and also compare blood plasma steroid concentrations of treatment groups as described for objective 2).

Objective 2: At the beginning of gonad recrudescence, blood will be sampled from the caudal vein monthly in Peruvian aquaculture conditions. Twenty to thirty fish will be sampled. Estradiol, testosterone, 11-ketotestosterone and 17,20 β-progesterone will be determined by radioimmunoassays (Ottobre et al., 1989; Dabrowski et al., 1996). Validation of all steroids for *Colossoma* has already been completed at OSU in a rigorous fashion using blood collected from broodstock maintained there. To compare mean steroid concentrations, as well as patterns of steroids over time, broodstock fish will be individually implanted with electromagnetic tags (PIT-tags, Biosonic, Seattle, WA). This method proved to be very reliable with *Colossoma* the last two years in Ohio. At the time of final maturation (normal spawning time in Peru is late November/early December), frequency of blood sampling will be increased to weekly intervals. At the time of hormonal treatment (Chellappa et al. 1996), blood sampling frequency will be increased to 6-12-h intervals, although different individuals will be used. No mortalities of 4-6-kg *Colossoma* occurred at OSU during the 1997 intensive sampling of blood and gametes.

Objective 3: The spawning season for *C. macropomum* and *P. brachypomus* in Peru is October–February. Pituitary extracts from various species have been used to induce ovulation, but with varying degrees of success (Campos, 1993). GnRHa (LHRHa) has been successfully employed using one or more dosages in the 0.5 to 10 µg/kg range (Campos, 1993). HCG has been used sporadically, generally at very high dosages (3.0 IU/g). The relative efficacy of GnRHa and hCG will be compared at three dosages (1.0, 5.0, 10.0 µg GnRHa/kg; and 1.0, 2.0, and 3.0 IU hCG/g). Conceptal® is locally available and will serve as the GnRHa commercial source. Chorulon® is internationally available, and will be the hCG commercial source. Peruvian aquaculturists routinely inject these hormones directly into the bloodstream, usually at the heart in a single injection. Hatching time occurs within 1-18 h post-injection. A second injection at half the dosage is used on fish that do not spawn within 24 h. Gametes will be stripped and fertilized using the dry method. Embryos will be incubated in MacDonald jars at 26 to 28°C. Fry hatch in less than 24 h and are stocked in ponds treated with chicken manure (0.1 kg/m^2/15 d) and triple superphosphate (30 g/m^2/15 d) (Ascon, 1988). Relative spawning success of the two hormones and three dosages of each will be compared in terms of ovulation response (degree hours) and hatching success (degree hours and percent hatch).

Site: Iquitos, Peru

Facilities: IIAP Quistachocha hatchery

Culture Period: N/A

Stocking Rates: 1.0 g eggs/L (hatching jar)

Water Management: Filtered well water

Other Inputs: N/A

Test Species: C. macropomum and/or *P. brachypomus*

Sampling Plan: 5 female fish for each dosage and for each hormone will be injected (30 fish total of each species, if both available).

Objective 4: Additional densities of *C. macropomum* and/or *P. brachypomus* will be compared in yield trials based on Eighth Work Plan results in which 3,000 and 4,000 fingerlings/ha are being tested. This study will also serve as the yield trial for the cost-of-production objective.

Site: Iquitos, Peru

Pond Facilities: IIAP Aquaculture Center (9 ponds of about 0.3 ha each).

Culture Period: 6-month grow-out

Stocking Rates: 3 ponds/stocking rate

Water Management: Fertilization with chicken manure (0.1 kg/m^2/15 d); triple superphosphate (30 g/m^2/15 d).

Other Inputs: Feed pellets (30% CP)

Test Species: Colossoma macropomum and/or *Piaractus brachypomus*

Sampling Plan: Bi-weekly; commencing week 7 post-stocking, samples of fish (N = 50) will be weighed to adjust food rations; at harvest, the following values will be calculated: survival (%), specific growth rate, standing crop at harvest, condition (K), and feed conversion efficiency.

Objective 5: Capital costs, as well as fixed and variable-operating costs, will be estimated based on the density study conducted for Objective 4. Estimates for a break-even analysis will be determined. The information on production costs will be obtained by tracking costs, feed conversions, etc. of various inputs in the study described in Objective 4.

Statistical Methods

Data values will be analyzed by one-way analysis of variance (ANOVA). Appropriate transformations will be made where necessary. If significant differences among treatment means are found, the appropriate post-hoc test will be employed to determine where the differences lie. The accepted level of significance will be 0.05.

Regional Integration

Research efforts being proposed are logical initial steps toward developing sustainable aquaculture in the region. Research needs were identified with considerable input from in-country scientists and agency administrators. The research will benefit the entire region by providing pertinent information on broodstock nutrition, reproduction, stocking densities, and cost of production.

Schedule

Yield trials: February to November 1999; February to November 2000
Nutrition and endocrine studies to be carried out from October 1998 to January 1999; October 1999 to January 2000.

Final Report Submittal

June 2000

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Kenya Research

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Fish Yields and Economic Benefits of Tilapia/*Clarias* Polyculture in Fertilized Ponds Receiving Commercial Feeds or Pelleted Agricultural By-Products

Feeds and Fertilizers Research 2 (9FFR2)/Experiment

Objectives

1) To compare the most profitable rice bran-chemical fertilizer treatment from a preceding experiment with a local commercially available poultry feed and a pelleted lower-cost feed without fish meal and formulated for fish.
2) To assess the relative contribution of natural food to the growth of fish receiving sub-optimal protein feeds.

Significance

Commercial fish culture in developed countries generally achieves greatest profits when high quality, nutritionally complete feeds are used to produce high fish yields. This strategy is often impossible or inappropriate in countries where high quality feedstuffs are limited. In Africa, nutritionally complete diets for tilapia are very expensive. However, poultry diets and some purchased inputs, such as brans, can be used to intensify fish production in ponds. Disadvantages are that commercial poultry rations are not nutritionally balanced for fish, containing more digestible energy per unit of protein than recommended for fish, and brans are nutritionally deficient and often unconsumed by the fish due to small particle size. Pelletizing reduces feed losses, especially when multiple ingredients are included in the formulation. There is a clear need to develop feed/fertilizer combinations that are appropriate for fish farming in Kenya (Ngugi and Wangila, 1996). Lower quality pelleted feeds formulated specifically for tilapia, combined with fertilization regimes to increase the availability of natural food organisms, may be an economically appropriate approach for intensification of tilapia culture in Africa.

This research is feasible in Kenya. Poultry diets and dairy meal are sometimes used in Kenya to feed tilapia. There is one commercial fish feed manufacturer in Kenya. They make trout feeds and small amounts of tilapia feeds and are willing to formulate other feeds.

Fertilization is often practiced to increase the abundance of natural food organisms in fish ponds receiving nutritionally deficient feeds. Assessment of the relative contribution of natural food organisms to fish growth in fed ponds would be a valuable contribution in the development of management practices involving nutritionally incomplete feeds. This assessment can be accomplished by analyzing the stable isotope ratios of N and C in the natural foods, feeds, and fish flesh.

Anticipated Benefits

Collaboration with a local feed manufacturer can lead to a viable partnership with private enterprise to develop the most cost-effective tilapia feed for ponds in Kenya and the region. The development of cost-effective feeds in Africa may increase the profitability of fish farming in the region and stimulate commercial aquacultural enterprises. The production of natural food organisms in feed-fertilizer management practices is often highly variable across sites; the need to evaluate this more intensive fish production practice under different environmental conditions would likely stimulate future intra-regional collaboration.

Research Design

Standard CRSP protocols will be used in all pond experiments.

Location of Work: CRSP research ponds, Sagana Fish Farm, Sagana, Kenya.

Pond Facilities: Three treatments will be replicated in four ponds of $800 m^2$ each:

Treatment 1: most profitable treatment from the Eighth Work Plan, Activity KR3, in which combinations of rice bran and chemical fertilizers were tested

Treatment 2: a locally available pelleted poultry feed (most likely candidates are dairy weaner or poultry finisher pellets, based on cost, availability and nutritional value) plus the fertilization regime of Treatment 1.

Treatment 3: a formulated pelleted feed, containing about 20% crude protein and 9 to 10 kcal of digestible energy per g of crude protein plus the fertilization regime of Treatment 1. The feed in Treatment 3 will contain no fish meal and will be developed in consultation with the local fish feed manufacturer. The primary ingredients of this feed will be full-fat soy cake, rice bran, maize germ and wheat bran.

Culture Period: Six months

Stocking Rates: All ponds will be stocked at densities of 30,000 fish/ha: (90% sex reversed male *Oreochromis niloticus* and 10% *Clarias gariepinus*). These proportions were used successfully in previous CRSP experiments. Average weight at stocking: 15 g for tilapia and 5 g for *Clarias*. Twenty-five tilapia will be stocked in cages in each pond and will not be fed. (These fish will be analyzed for C and N isotopes and compared to those in the open pond, which have access to feed.)

Test Species: *O. niloticus* and *Clarias gariepinus*

Nutrient Inputs: Feed and fertilizer treatments as described above. Feeding rates will be based on estimated biomass of tilapia: 3% of body weight per day for fish less than 50 g, 2% of body weight per day for fish between 50 and 150 g, and 1% of body weight per day thereafter. (These rates are approximately the same rates as used in Thailand when fish were fed at 50% satiation.) Depending on the diet composition, it may be necessary to feed the experimental animals a diet that is isotopically distinct from those diets to be used during the experiment for a period of 2–3 months prior to initiation of the trial.

Water Management: Water lost through seepage or evaporation will be replaced weekly.

Sampling Schedule: Fish will be sampled monthly by seining to determine average weight for adjustment of feeding rates. Thirty fish of each species will be weighed and measured individually to obtain standard deviations. Water quality will be sampled according to standard CRSP sampling protocol. For isotope analyses, fish and food/feed samples will be collected at stocking, at harvest, and every 1.5 to 2 months after stocking. Gut contents of caged and free-swimming fish will also be collected at least twice to back up results of the fish tissue analyses. This will be done in case the prepared feeds are not isotopically distinct from the natural foods, and visual distinction of consumed food items is necessary. Harvest data will include fish yield (fish weights & fish numbers), water quality data, percent of market-size fish, and reproduction. Proximate analysis of fish and feeds also will be conducted using standard methods.

Statistics: Null hypotheses: The nutrient input regimes tested do not differ significantly with respect to their effects on fish growth, yield, feed conversion, or C or N stable isotope ratios. Statistical analyses: ANOVA

Regional Integration

Fertilizer-enhanced natural pond productivity, required to supplement the nutritional deficiencies of lower quality fish feeds, will likely be variable within a region. The most profitable feeding-fertilizing regime under the test conditions at the Sagana station may be substantially more or less profitable at other sites. Assuming that the expected increased fish yields generate regional aquacultural interest, this topic could be a focus for future regionally integrated research.

Schedule

July to November 1999

UAPB personnel will arrange for isotope analysis of samples, interpret isotope data and compare it to production data from feeding trials in Kenya (in collaboration with Kenyan scientists and students), and conduct proximate analysis of fish and feed samples. Kenyan and Auburn personnel at Sagana fish farm will conduct the feeding trial, collect and process samples for isotope analysis and collect production data. Some proximate analysis also may be conducted at Sagana (depending on availability of equipment).

Report Submission

Final report: June 2000

References

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Use of Pond Effluents for Irrigation in an Integrated Crop/Aquaculture System

Effluents and Pollution Research 1 (9ER1)/Experiment

Objectives

1) Evaluate pond effluents as a source of irrigation water for maize and soy.
2) Assess the ability of pond effluents to increase soil test phosphorus and increase phosphorus uptake of maize and soy.
3) Produce integrated water and nutrient budgets for fish ponds/crop systems.

Significance

The second planting season in Kenya begins in about October and continues through December. Rains can be quite unreliable and in the last decade, second season crops have failed 40 to 60% of the time. Farmers typically plant early-maturing maize which matures in 90 days to reduce their risks of crop failure. These varieties typically provide only 50% of yields of the later maturing varieties but they are less prone to losses from drought. Irrigation can provide the water needed to allow farmers to plant high-yielding crops. Farming fish in irrigation reservoirs can provide additional revenues. The majority of irrigation in Kenya is practiced by small scale irrigators (16,700 ha) versus 9,023 ha for large irrigation schemes. Eighty percent of Kenya's land falls in the ASAL regions (arid and semi-arid). The Ministry of Land Reclamation, Regional and Water Development seeks to promote multiple uses of irrigation waters (Government of Kenya, 1996).

Aquaculture in Kenya is practiced in water retention reservoirs and in fish ponds. While the former make up a greater surface area, the latter tend to be much more productive. One reason is that farmers who use reservoirs for crop irrigation are hesitant to add fertilizers to reservoir ponds because there are no guidelines for this and no benefit to crops has been demonstrated. On the other hand, farmers who wish to intensify their aquaculture production may be interested in knowing that the water from their ponds can have beneficial effects on their crops in addition to the advantages arising from having a source of water. Soils in much of Kenya and eastern and central Africa tend to be very limited in available phosphorus and have high phosphorus fixing ability. Effluents from fertilized ponds can have high phosphorus concentrations and be potential sources of pollution. It is therefore advantageous to use one system's waste as another system's resource.

Fisheries Department staff in Kenya tend to function on their own with little input and advice from the agriculture sector because each answers to a different Ministry. A collaboration between agriculture researchers and fish-farming researchers can help each sector understand and incorporate the other into their line of thinking. Working through university professors assures that experiences gained by professors will be transferred to numerous students along the way. Kenyan counterparts will be instrumental in carrying out the experiment, interpreting the results, and transferring the information to user groups.

Anticipated Benefits

The value of this research is in the demonstration that fish farming and irrigation can be complementary as opposed to competitive. Water requirements for ponds at Sagana will be known from previous work on water budgets. This trial will demonstrate some possible beneficial effects of fertilizing water retention reservoirs that are used solely for irrigation. It may also show the benefits of passing pond effluents through farm land. Comparative water budgets and cost/benefit analyses for fish culture and irrigation will allow irrigation planners to assess the benefits of adding fish culture activities to irrigation schemes. (A rough estimate of cost of fertilizers and anticipated fish yields was made to make sure fish yield can more than cover the cost of fertilizers applied to ponds.)

Activity Plan

Location: Sagana fish farm, Kenya; Faculty of Agriculture, Dept. of Soil Science, University of Nairobi (greenhouse trials)

Methods: The experimental design will be a split plot with levels of P in pond effluents as main plots.The main treatment will be P added to water used for irrigation. Different P levels in the pond water will be obtained through fertilization with P at different rates. Although pond soils at Sagana are over 80% clay and have a high P-fixing capacity, they will have been saturated with P prior to use for this experiment.

Treatment 1:	no irrigation.
Treatment 2:	irrigation with canal water (no added N or P). Canal water contains less than 0.1 mg/L total P.
Treatment 3:	irrigation with pond water receiving 2 kg/ha/wk P.
Treatment 4:	irrigation with pond water receiving 5 kg/ha/wk P.
Treatment 5:	irrigation with pond water receiving 8 kg/ha/wk P.
Treatment 6:	irrigation with pond water receiving 11 kg/ha/wk P.

This will result in 6 x 3 reps = 18 plots, of about 25 m^2 each. Each main plot will be split into subplots consisting of crop varieties recommended for the Sagana area:

a) Late-maturing maize, variety hybrid 511 or 512
b) Soy ("Andre-25" if available; otherwise "Blackhawk")
c) Maize/soy intercropping

This results in 18 plots x 3 subplots/plot = 54 subplots. Each subplot will consist of four rows of 5 meters each. The two outer rows will be guard rows and will serve for pre-harvest biomass sampling. The two inner rows will be used for yield measurements.

Pre-harvest biomass sampling: maize ear leaf samples at silking, and uppermost mature leaves of soy at the R1 (first flower) stage of growth. Maize whole plant samples and soy whole plant samples at R1 will also be collected. Leaf samples of maze and soy will be analyzed for P and other nutrients to determine sufficiency/deficiency. Whole plant samples will be used to assess mid-season nutrient uptake.

Harvest Sampling: grain yield and grain subsamples for protein and nutrient analyses.

Soil Sampling: surface soil (15 cm) will be sampled prior to planting and after harvest for analysis of organic matter, extractable P (Olsen method, depending on pH), and extractable nitrate and ammonium.

Subplots (except Control 1) will be irrigated by the furrow method, following standard irrigation recommendations determined by weekly sampling in all subplots of that treatment. Tensiometers will be used to assess soil moisture. However, if earlier trials on drip irrigation demonstrate that it is feasible for these crops and with algae-rich water, drip irrigation will be used instead of furrow irrigation. The reason for favoring drip irrigation is that the irrigation scientists currently recommend drip methods as the most water efficient. Planting and weeding will be done according to accepted methods for this area.

Culture period for crops will be 140 days for the maize; less for soy.

Ponds: A total of 12 ponds of 800 m^2 each will be used.

Pond Fertilization: Ponds will be limed prior to filling at the rate determined necessary from following the general lime requirement (Boyd, 1994). DAP will be the sole source of P. Nitrogen will be provided at a rate determined to be optimal for this site and for warm season from previous CRSP research, probably about 20 kg N per ha per week. Nitrogen will be provided by DAP and urea.

Water Movement: Water required for irrigation is anticipated as follows: At 500 mm water added as irrigation, and 75 m^2 total plot area per treatment use 0.375 m^3 or 375 liters per treatment. If furrows are used, more than double this amount will be needed. The current drip irrigation method recommended involves use of a 70-liter plastic barrel (cost $8 each) and hose pipe. The barrel can be filled by siphoning from the ponds. Given the total pond volume (3 ponds x 800 m^2/pond x 0.8 m average depth = 1,920 m^3), water use will be minimal.

Sampling: Water, soils, fish, and crops will be sampled in order to make nitrogen and phosphorus budgets for the pond/crop system. Data needed for N and P budgets will also serve to make water budgets.

A complete weather station with dataloggers will be in place at the site to provide max and min air temperature, relative humidity, solar radiation (as PAR and watts), precipitation, and wind speed data. An evaporation pan is in use at Sagana station.

Fish sampling: Fish will be sampled by seine net at biweekly intervals to measure growth; approximately 10% of the initial stock will be seined up, counted, and weighed en masse. Ponds will be harvested by draining upon completion of the trial.

Concurrent greenhouse trials using different soils from Kenya will be used to assess effects treatments used in the field experiment could have on other soils. Water from the ponds will be used.

Statistical Design: null hypotheses:

1) Irrigation does not increase crop yields.
2) P level in water used to irrigate does not affect crop yields.
3) There is no difference in soil-P following one or two seasons of irrigated maize or soy production.

Regional Integration

Making water budget information available to policy makers is an area of activity recommended in the regional plan for Africa. The test crops are grown throughout southern, eastern and central Africa; maize is a staple from southern Africa to Kenya and soy is a rather new but promising crop being promoted by several agriculture programs. A soy irrigation scheme has just started about 30 km from Sagana. Soy is an important ingredient of animal feeds and its low production level has been a leading cause of high costing animal feeds in Africa.

Schedule

Pond filling and stocking will be done in early November 1998. It is anticipated that ponds will be drained after about 5 to 6 months (April 1999) Planting of crops will occur in October and crop harvest will be in January to February 1999. This schedule should provide the water necessary at the most critical times for crop production. The experiment will be repeated from May to October 1999. This is often not a rain-limited season but expectations are for a drought in 1999. Planting will be done in mid-May and harvest is usually in August. Report on first harvest due May 1999. Report on second harvests due February 2000.

References

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On-Farm Trials: Evaluation of Alternative Aquaculture Technologies by Local Farmers in Kenya

Appropriate Technology Research 1 (9ATR1)/Study

Objectives

1) To collaborate with local fish farmers in the testing of technologies developed through PD/A CRSP research.
2) To demonstrate improved management techniques to extension officers and farmers.
3) To teach simple methods for evaluating costs and benefits to farmers and extension agents.

Significance

Fish farmers throughout Kenya, as well as the extension agents who serve them, suffer from a lack of information about good pond management practices and technology alternatives that may be available to them. Some of the major consequences of this are that many current farmers do not achieve good fish production in their ponds, other farmers become “inactive,” and potential farmers avoid going into fish culture because its profitability has not been convincingly demonstrated to them. These and other factors combine to result in typical low productivity from Kenyan fish ponds.

Once current production practices and on-farm conditions are known, research conducted by the PD/A CRSP at Sagana Fish Farm or other research centers helps to identify alternative management practices and technologies that may be suitable in the region, but it cannot be assumed that results obtained under controlled experimental conditions are directly transferable to the farm. On-farm testing is therefore a logical step in transferring research-based technologies to the farm. On-farm testing of various alternatives allows farmers to assess their costs and benefits under local conditions as well as to receive instruction and training in basic pond management skills. The conduct of such trials also provides opportunities for project personnel to work with and train the fisheries extension officers who are involved in the trials at the various locations, thus complementing the training they will receive through a training activity proposed as part of this Regional Plan (“Aquaculture Training for Fisheries Officers in Kenya”).

Anticipated Benefits

Farmers will be able to evaluate and compare alternative technologies tested in their own ponds, then apply those technologies that are most appropriate under their specific conditions. Farmers and extension officers will receive on-the-job training and will gain skills in basic pond management practices. Farmers and extension agents will also learn to keep good records regarding the operation of fish ponds so that evaluations can be based on documented facts. Adoption of some of the technologies tested will in turn result in higher fish production and increased revenues from fish sales for participating farmers. Neighboring farmers may also adopt the new technologies or apply the improved management practices they have observed at the trial sites.

Research Design

Location of Work: Selected farmers’ ponds in Central Province (Kenya) during Year 1, and Western and Nyanza Provinces during Year 2. Ponds will be selected from Kirinyaga, Muranga, Nyeri, and Nyahandarua districts of Central Province. Western region districts will be determined during Year 1. Farmers will be selected according to their willingness and ability to participate, and on the basis of suitability of their ponds for this type of trial (see below). Communication with extension agents and prospective farmers has already been initiated to ensure that there will be sufficient time to identify participants and prepare for the trials.

Pond Facilities: Twenty to forty ponds will be selected for use in these trials. Ponds will be selected to meet the following criteria:

1) The owner is interested in participating in on-farm trials.
2) Surface area per pond of 100 m^2 minimum, and 1,000 m^2 maximum.
3) The pond is drainable.

4) Average water depth of the pond is 80 plus or minus 10 cm.
5) The pond is not prone to flooding.
6) Seepage from the pond is less than 10 cm per week.

Culture Period: Six months or less, provided fish reach a size considered marketable by the producer.

Stocking Rates: All ponds will be stocked with *Oreochromis niloticus* at 2 fish per m^2 and *Clarias gariepinus* at 2 fish per 10 m^2.

Test Species: *O. niloticus* (T), sex-reversed males of 10 g, or mixed sex, depending on treatment. *C. gariepinus* (C) of 5 g. All fingerlings will come from Sagana station.

Nutrient Inputs: Urea and DAP will be sources of inorganic N and P. Brans that can be used are maize bran, rice bran and wheat bran. Farmers will provide a sample of the bran for proximate analysis each time they purchase a new batch.

Treatment 1:	monosex T + C, with bran + inorganic fertilizer based on best treatment of the Eighth Work Plan, Activity KR3
Treatment 2:	same as Treatment 1 except with mixed-sex T
Treatment 3:	monosex T and C, with weekly additions of manure/organics at 500 kg TS/ha/wk
Treatment 4:	same as Treatment 3, except with mixed-sex T

Treatments 1 and 2 require daily feeding; Treatments 3 and 4 do not. Treatments 1 and 2 usually require capital expenditures, while Treatments 3 and 4 probably do not. If possible and practical for producers, bran types used will be equally distributed across treatments 1 and 2. Treatments 3 and 4 are similar to current practices on farms but input quantities may be higher in this experiment.

Extension agents and farmers will participate in planning workshops prior to the beginning of the trials. Students currently involved in thesis work at Sagana or other sites near the workshop locations will also be invited to participate. These workshops will provide a forum for discussion of alternative management schemes, including definition of the manure/organic inputs to be used in treatments 3 and 4, and give farmers the opportunity to choose which input regime they wish to test. However, the selection of who will test monosex vs. mixed-sex fish will be done at random, because it is anticipated that most farmers will select monosex if given the choice. Pond management techniques and record keeping will be demonstrated at the pre-trial workshops. If possible, fisheries officers involved in CRSP-sponsored training courses (our "Aquaculture Training For Kenyan Fisheries Officers And University Students" activity proposed for this work plan) will make field visits to participating farms during the course of the trials. An evaluation workshop will be conducted after the trials to present the results to farmers and to discuss probable causes and economic consequences of the differences observed. Farmers' evaluations of the tested management schemes, including both pond productivity and economic aspects of production, will be solicited.

Water Management: Water lost from seepage or evaporation will be replaced weekly. Farmers will record the days on which they needed to add water.

Sampling Schedule: Ponds will be sampled monthly by seining. At the monthly sampling visit, a water sample will be taken to determine uncorrected chlorophyll *a*, total N, and total P. Water temperature loggers will be placed in selected ponds. Farmers will record:

- Secchi disk reading and water color - weekly
- input type and weight - as added
- pond water additions - weekly
- mortality - when observed
- expenses - weekly

Statistical Design: ANOVA and regression as appropriate.

Regional Integration

This study addresses the top three objectives listed in the regional plan for Africa (PD/A CRSP, 1997), including the investigation of alternative fertilizers and feedstuffs for ponds operated without supplemental aeration, the development of training activities that emphasize pond operation and management, and the collection of information that can be used to prepare enterprise budgets and business plans for fish production systems. During this Work Plan period the trials will be conducted in three of Kenya's eight provinces (Central, Nyanza, and Western). Similar trials may be conducted in other Kenyan provinces or in neighboring countries during subsequent Work Plan periods.

Schedule

Central Province Trials:

July to August 1999:	Survey farmers to select suitable participants
September 1999:	Conduct preliminary workshop
October 1999:	Begin trials
April 2000:	Trials concluded
May 2000:	Evaluation workshop

Western Region Trials:

January 2000:	Survey farmers to select suitable participants
February 2000:	Conduct preliminary workshop
March 2000:	Begin trials
September 2000:	Trials concluded
October 2000:	Evaluation workshop

Report Submission

Final report, Central Province trials: February 2001
Final report, Western Region trials: March 2001

References

PD/A CRSP, 1997. Working Paper on Regional Plans. July 1997. Pond Dynamics/Aquaculture Collaborative Research Support Program, Oregon State University, Corvallis, Oregon, 26 pp.

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Aquaculture Training for Kenyan Fisheries Officers and University Students

Adoption and Diffusion Research 3 (9ADR3)/Activity

Objectives

1) To increase the pond management skills of fisheries personnel currently involved in aquaculture extension activities in Kenya.
2) To enhance the research and extension capabilities of Kenyan university students likely to be employed in the aquaculture sector.

Significance

Production from capture fisheries in Kenya is approaching maximum sustainable yields, and the total catch of fish from Lake Victoria has stagnated over the last four years. Aquaculture production must be increased to compensate for the shortfall in overall fish production in Kenya (Government of Kenya, 1996).

Aquaculture in Kenya has evolved from a sport fishery on rainbow trout at the beginning of the century to predominantly small-scale subsistence farming, mainly using species that are endemic to the region such as tilapia. Culture of introduced species, such as common carp (*Cyprinus carpio*) and black bass (*Micropterus salmoides*), has had varying degrees of success. Tilapia have been under culture in static water ponds in Kenya since 1924, and the Nile tilapia (*Oreochromis niloticus*) is the main species cultured today (Ngugi and Wangila, 1996).

Annual aquaculture yield in Kenya had leveled off at about 1,200 metric tons by about 1990, less than 1% of Kenya's total annual fish harvest (Achieng, 1994). Reports on the status of aquaculture in Kenya vary, with the number of ponds ranging from 25,000 to 40,000. Productivity is low; average production from individual ponds in one province was estimated at less than 500 kg/ha/year. The key constraint to aquaculture production reported by fisheries officers in all provinces is a lack of adequate technical skills among fisheries officers, leading to the transfer of unproductive technology to farmers (CRSP Workshop, 1997). Training in fish handling and pond management skills for fisheries extension officers at several administrative levels will translate into better information for farmers and improved pond management and increased fish production in the country.

Aquacultural growth in the near future will be influenced by university students. Unfortunately, support of graduate students seeking specialization in aquaculture and guidance and support of students during the design and conduct of field studies in aquaculture are often limited due to financial constraints. Wangila (1996) points to the need to include more technical certificate and diploma and higher level training in training plans for the aquaculture sector.

Anticipated Benefits

This activity will provide fisheries extension workers with improved fish handling and pond management skills and lead to the dissemination of better fish culture information to the farmers. Short training courses will improve morale and technical confidence among extensionists. Linkages between research and extension activities in Kenya will be strengthened. Ultimately, better pond management by farmers will lead to increased fish production, increased farm income, increased amounts of fish available to communities and markets, and increased employment opportunities. Support and hands-on guidance of graduate students in aquaculture will strengthen their degree programs and ultimately help promote productive and sustainable aquaculture growth in Kenya and in the region.

Activity Plan

Fisheries Extension Officer Training

Training will be provided for fisheries extension officers at four levels: fish scouts, fisheries assistants, assistant fisheries officers, and fisheries officers. Fifteen 7-day training sessions will be organized as follows:

Training sessions will be conducted at provincial fish demonstration and farmers training centers, Ukwele Pastoral Centre (for the western region), Matuga Development Centre (coastal region), Bethany Catholic Centre (eastern region), or Sagana Fish Farm.

Trainees will be invited to apply for admission to the courses and their supervisors will be asked to recommend them. Two trainers will conduct each training session. The CRSP on-site researcher at Sagana will assure the course content is up to date and will provide some hours of instruction. The majority of the training hours will be undertaken by Kenyan trainers to be named. Subject matter to be covered will vary according to the service level and previous training of trainees, but will concentrate on basic pond management skills such as fish handling, fish feeding, pond fertilization and management, water quality management, and record keeping. Course notes will be printed and made available to selected farmers as well. Emphasis will be placed on practical field work, with only about 25% of training time being spent in a classroom setting.

Student Stipends
Short-term stipends will be provided to support research conducted by Kenyan university students at the Sagana Fish Farm. Research must be part of an aquaculture-related degree program, and the subject area must be approved by the CRSP US researcher at Sagana. Subject areas will include topics such as fish production and feeding, N and P budgets, and phyto- and zoo-plankton dynamics. Stipends will normally cover periods of six to twelve months each.

Full Scholarships
Full two-year scholarships will be provided to two selected Masters level students during the period of this Work Plan. Candidates will be invited to apply for these scholarships, and their selection will be based criteria such as academic qualifications, proposed research topics, recommendations from undergraduate instructors, previous performance in the extension sector, and interviews. The main objective is to train personnel who can subsequently function as links between the research and extension sectors. Their future roles will be to evaluate research results and translate them into extension messages.

Regional Integration
This activity directly addresses Objective 2 of the PD/A CRSP Regional Plan for Africa (PD/A CRSP, 1997), which is "to assist in the development and conduct of aquaculture training courses and programs, with emphasis on pond operation and management."

Schedule
Training sessions will be held approximately monthly, beginning about December 1998 and finishing up around the end of November 2000.

Report Submission
The final report for this activity will be submitted in March 2001. Training courses and total hours will be summarized in quarterly reports.

References
Achieng, A.P., 1994. Aquaculture development and research in Kenya. In: Andre G. Coche (Editor), Aquaculture Development and Research in Sub-Saharan Africa, National Reviews. CIFA Technical Paper No. 23 Suppl. Food and Agriculture Organization of the United Nations, Rome, pp. 169-207.
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Wangila, B.C.C., 1996. Fishery training needs. In: Fisheries for Sustainable Development. Technical Report No.1, Department of Fisheries, Moi University, Eldoret, Kenya, pp. 58-64.

Establishment of Companion Sites in the Africa Region

Adoption and Diffusion Research 4 (9ADR4)/Activity

Objectives

1) To identify and establish one or more companion sites for the Africa Region.
2) To define and implement investigations at the companion site in support of PD/A CRSP and companion site goals.

Significance

Research at an African site has been a major component of the PD/A CRSP since its beginning in 1982, first at the Rwasave Fish Culture Station in Rwanda, and since early 1997 at the Sagana Fish Farm in Kenya. During this 15-year period, research efforts have centered almost entirely around the Rwasave Station and nearby areas where on-farm trials were conducted. Under the CRSPs new five-year grant (1996-2001), part of our mandate is to regionalize our efforts, extending the validity and benefits of CRSP research and training beyond the immediate vicinity of prime sites and into surrounding regions and countries. Contact and communication with scientists at aquaculture research institutions and extension program personnel is one way to achieve such regionalization, and these types of activities are planned as part of the "Regional Outreach" activity outlined elsewhere in this Work Plan.

Verification of CRSP research results and strengthening of institutions can also be achieved through the complementary activity of initiating collaboration and supporting research at companion sites. This type of effort was promoted in the PD/A CRSP Continuation Plan and accepted by the granting agency as a valid approach. In this activity we will establish at least one companion site. The first such site will probably be in East Africa, but a second site may also be established in West Africa, thus extending the effectiveness of the CRSPs effort in the Region. The selected Companion Site(s) will be ready for "investigations" such as holding CRSP protocol workshops and conducting experiments, which will be included in a subsequent CRSP Work Plan, if any.

Anticipated Benefits

Africa is the source of great genetic variation in the *Oreochromis* genus. The aquacultural potential of many *O. niloticus* strains remains untested. In order to reduce the risk of genetic contamination, a logical first step in strain evaluations is performance testing in the region where a strain is endemic. Companion sites provide great opportunities for carrying out this process.

The CRSP Central Database will be broadened through the inclusion of data from additional sites in Africa. The validity of CRSP-sponsored research results can be verified for areas away from the prime site. Companion site researchers will benefit from data collected during the course of experiments. Improved fish farming methods resulting from the experiments will be available for adaptation by extension services and adoption by fish farmers in the areas around companion sites. Ultimately, fish farmers in new areas will experience increased fish yields and greater amounts of fish will be available for consumption in communities and markets in those areas.

Activity Plan

During Year 1 of the Ninth Work Plan, contacts previously made with officials at potential companion sites will be reestablished and earnest discussions on the possibility of collaborating with the CRSP will be initiated. Discussions will occur via telephone, fax, or email as much as possible, but a site visit by the US researcher posted at Sagana or one of the US PIs may also be needed. Discussions will include evaluations of the physical facilities at each site and their suitability and availability for CRSP research, the availability of qualified researchers for carrying out experimental work, the potential for student involvement in the research, and the amount of site development that would be needed to prepare a given site for CRSP experimental work. Areas of research that will be mutually useful, i.e., that would not only advance lines of investigation already being pursued by the CRSP but also address needs of fish farmers in the vicinity of the companion site, will be identified. Training needs of potential companion sites will be assessed so that when possible these can be addressed in future work plan activities. If the CRSP researcher at the Prime Site in Africa (Sagana) and the project's Principal Investigators agree that a site is suitable and that both the regional goals of the CRSP and the needs of local fish farmers would be served by a collaborative effort there, a proposal to collaborate will be put forward and the process of establishing a Memorandum of Understanding (MOU) will be initiated.

The sites that will be actively investigated as possible companion sites include Bunda College of Agriculture (Malawi), Kingolwira Aquaculture Center (Tanzania), and Akosombo Aquaculture Research and Development Center (Ghana). Additional sites may also be investigated. Criteria for the selection of suitable companion sites are identified in the CRSP grant (*Continuation Plan*, pages 69-72). Companion sites need not be in the same country as a prime site, but should meet as many of these criteria as possible: proximity to the regional prime site, shared or complementary environment (with the prime site), potential for future development, proportionally reduced investment requirements, low personnel and operation costs, integrated research objectives, and healthy political relationships between the respective governments (prime and companion site countries).

Once a suitable MOU is established, composite soil and water samples will be analyzed to characterize the site and a standardizing investigation that is of interest to companion site researchers and consistent with PD/A CRSP goals will be planned and initiated. Treatments suitable for this type of investigation include one of the bran/fertilizer treatments described in the Eighth Work Plan, Study KR3, or one of the follow-on treatments proposed for the Ninth Work Plan feed trial ("Fish Yields and Economic Benefits of Tilapia/*Clarias* Polyculture in Fertilized Ponds Receiving Commercial Feeds or Pelleted Agricultural By-Products").

Regional Integration

This activity was specifically mentioned as a goal for the Africa Project in the Regional Plan for Africa (PD/A CRSP, 1997). Potential sites were named, with the intent of continuing to explore possibilities and make recommendations leading to the establishment of at least one companion site in Africa.

Schedule

Year 1: Identify and establish a companion site.
Year 2: Initiate the first investigation at the companion site.

Report Submission

Activities undertaken will be reported on annually in PD/A CRSP Annual Reports.
A final report will be submitted March 2001.

References

PD/A CRSP, 1997. Working paper on regional plans. July, 1997. Pond Dynamics/Aquaculture Collaborative Research Support Program. Oregon State University, Corvallis, Oregon, 26 pp.

Regional Outreach in Africa

Adoption and Diffusion Research 5 (9ADR5)/Activity

Objectives

1) To promote the dissemination of information emanating from PD/A CRSP research results.
2) To learn about fish culture practices and research priorities and activities in Kenya and neighboring countries in Africa.
3) To encourage the establishment of regional linkages between research and extension programs in Africa.

Significance

A serious constraint to increasing aquaculture production through research in Africa is linking research with extension services. Research conducted at the former PD/A CRSP research site in Rwanda has still not made its way out to the African researchers and extensionists because of low participation in regional meetings. Research conducted at the new CRSP site at Sagana Fish Farm will face the same fate if intra-regional participation is not actively promoted. The Fisheries Society of Africa has begun holding conferences, providing an excellent opportunity to communicate research results from current and previous CRSP activities, to meet and encourage other African researchers, and to promote communications between research and extension organizations. Communication and collaboration between countries in the Southern Africa Development Community (SADC) region includes an annual meeting of SADCs Inland Fisheries Technical Cooperation Unit based in Lilongwe, Malawi. Attendance at these annual meetings and frequent mailings of publications to the extension services are cost-effective means of communicating research findings to farmers. Pre-meeting contacts and planning can be used to foster greater participation at such meetings.

Anticipated Benefits

Contact with researchers and extension personnel in Kenya and in other countries in the region will result in a better understanding of research needs and enhanced research-extension linkages. Extension services in Kenya and other African countries will benefit by being more closely linked with research institutions and African researchers will have an enhanced understanding of research needs. Ultimately, fish producers throughout the region will benefit, because these linkages will enable extension services not only to more easily convey farmers' needs to researchers, but also to extend new research results back to the farmers.

Activity Plan

1) Project personnel will attend and make presentations at meetings of the Southern Africa Development Community (SADC) Inland Fisheries Sector Technical Coordination Unit and at annual meetings of the Fisheries Society of Africa (FISA). Up to five people may attend such meetings, including the host country PI, the host country RA, the on-site CRSP researcher, and two additional attendees from the region, including students when appropriate. The selection of attendees will be made by joint decision of the PIs AND RAs of the project. Attendees will be actively involved project people who can share the results of CRSP experiments, studies, or activities by making presentations; they will also use these meetings to meet with regional colleagues to examine the feasibility of producing extension publications useful to the region.
2) Attendees will learn about fish culture activities and research and extension priorities in the region by attending presentations made by others and by participating in special sessions arranged for these purposes at the conferences (see also item 4, below).
3) The project may also provide partial financial support for participation in regional meetings by key colleagues who have something to present or can make a substantial contribution at special planning and coordinating sessions (see also item 4 below) but who might otherwise be unable to attend.
4) Project personnel will become actively involved in the organization of regional meetings, in pre-meeting coordination, and in efforts to develop and implement plans to increase participation in these meetings. They will endeavor to work with the organizers of regional meetings to ensure that

opportunities are provided (e.g., through inclusion of special sessions or workshops in conference programs) for exchanging information and for holding in-depth discussions on regional research and extension priorities or on creating linkages for collaboration on research and extension efforts.

Regional Integration

This activity directly addresses the second main goal identified in the Regional Plan for Africa (PD/A CRSP, 1997), which is "to encourage and support cooperation, communication, and technology transfer among research and extension institutions in the region."

Schedule

FISA and SADC meetings will be attended each year that they occur (meeting dates to be announced).

Report Submission

A report of meetings attended or other outreach efforts undertaken under this activity will be submitted annually during the Work Plan period.

First annual report:	June 1999
Second annual report:	June 2000
Final report:	March 2001

References

PD/A CRSP, 1997. Working paper on regional plans. July, 1997. Pond Dynamics/Aquaculture Collaborative Research Support Program. Oregon State University, Corvallis, Oregon, 26 pp.

Philippines Research

Collaborating Institutions
Central Luzon State University
Remedios Bolivar

University of Hawaii—Lead US Institution
Chris Brown
James Szyper

Reduction of Rations below Satiation Levels

Feeds and Fertilizer Research 3 (9FFR3)/Study

Timing of the Onset of Supplemental Feeding

Feeds and Fertilizer Research 4 (9FFR4)/Study

Workshops and Production of Improved Extension Materials

Adoption/Diffusion Research 6 (9ADR6)/Activity

Background
Collaborative work involving the Freshwater Aquaculture Center at Central Luzon State University (FAC/CLSU) and the University of Hawaii (UH) began as part of the Pond Dynamics/Aquaculture CRSP Sixth Work Plan in 1991. Originally started as a relatively small-scale ancillary component of the Thailand project, this collaboration has grown steadily and the scope of work has increased to the degree that FAC/CLSU is now considered to meet all requirements for selection as a CRSP prime site in the Philippines.

In support of this change in the status of FAC/CLSU within the PD/A CRSP program, socioeconomic studies carried out by Molnar et al. (1994) concluded that Central Luzon is receptive to the adoption of new technology and should therefore benefit from a concentration of CRSP activities in the area. Among other observations, it was noted that aquaculture methods and concepts consistent with ideas developed within the CRSP program have been advocated by the Philippine Bureau of Fisheries and Aquatic Resources (BFAR) for many years. CRSP activity, however, has led to a demonstrable increase in attainable yields of pond-grown tilapia.

Research at the CLSU site to date has concentrated on pond fertilization protocols and examination of strain-related differences in growout performance among tilapia. The cumulative experience of CLSU and UH participants has led us to the conclusion that despite improved yields, optimization of the pond fertilization strategy and pond management methods leaves many of the practical problems that limit the intensification of tilapia production in the Northern Philippines unresolved. Studies conducted by the CRSP in Thailand suggest that supplemental feeding of tilapia in fertilized ponds may induce growth advantages over feeding or fertilization alone (Diana et al., 1994a). Our goal for the proposed project is to explore the possibility that low-level feeding or feeding during only part of the grow-out phase may promote production in a cost-effective manner that will be readily acceptable for fish farmers in the Philippines.

Work on genetics of *Oreochromis niloticus* hybrids is the subject of a continuing effort under the direction of the International Center for Living Aquatic Resource Management (ICLARM) Genetic Improvement of Farmed Tilapia (GIFT) project. This project is also being carried out at CLSU. We will remain closely attuned to this project, assisting to the degree that we can, and integrating their results with our own as our project progresses. The UH/CLSU collaboration has also involved "genetically male tilapia" work conducted at CLSU by the U. Wales/Swansea project.

One specific problem requiring attention is the production of monosex tilapia, which has been problematic at the CLSU hatchery despite apparently adequate training and background knowledge in the technology. Although this is not a primary focus of our research, we will troubleshoot this and other problems that arise at CLSU to the best of our ability.

Research Problem

A variety of farming methods are currently in practice in the Philippines, ranging from family-scale subsistence production to commercial-scale farming. It is generally true that all tilapia produced can be sold, and recently markets for larger tilapia at premium prices have developed. For these reasons, we feel that the major project emphasis should be on a practical component of production at this time, and one which can be integrated into farming that takes place on a range of different scales.

The proposed work aims to contribute to optimized intensification of tilapia farming through a combination of on-farm and on-station development of:

1) integrated fertilization and supplemental feeding practices;
2) documentation of a range of applicable levels of management intensity in terms of stocking density and the appropriate management of dissolved oxygen (e.g., aeration); and
3) improved materials and practices for wider dissemination of results.

It is has recently become generally profitable to grow tilapia in ponds in central Luzon, to the degree that intensification beyond the traditional fertilization strategy (i.e., use of prepared feeds, increased stocking densities) has begun. A notable difference from the earlier traditional market condition is that there is now a substantial price premium for tilapia of larger sizes (at least 250 to 300 g/fish). Production of this product involves feeding the fish during at least some portions of the production cycle. However, both researchers and farmers are aware that feed costs are high and can compromise profits. At present, practices are variable and the progress of intensification on particular farms may be disorderly and of a trial-and-error character. Technical guidance would smooth and hasten progress toward optimization.

Concurrently, there are and will remain small farms which are unlikely to follow optimal intensification methods, but which may nevertheless benefit from recommendations developed by the PD/A CRSP. The logical progression from the CRSPs development of efficient fertilization protocols is to integrate options of stepwise intensification by way of supplemental feeding, and other management practices. Therefore, pond production of tilapia and other fishes in central Luzon, already one of the Philippines' most productive inland regions, would benefit from the dissemination of a comprehensive approach to a range of intensity levels, all having a sound theoretical and empirical basis, and all having been demonstrated under local conditions. This has been done, though largely on-station in the case of feeding experiments, by the Thailand CRSP project (Szyper et al., 1995; Table 1).

Table 1. Experimental Stages of the PD/A CRSP (in Thailand: from Szyper et al., 1995).

Stage Number	Fertilizer Inputs	Fertilization (kg/ha/week)	Approximate Fish Yield (kg/ha/year)
1	Phosphorus, inorganic	2 units	800
2	Chicken litter	500 units (dry basis)	5,000
3	Inorganic combination	N: 28 units; P: 7 units	8,000
4	Fertilizer & feed	Stage 3 + pellets @ 50% satiation	22,000

Objectives

The overall goal of the proposed work is to develop and disseminate improved pond management options for intensified production of tilapia by farmers with different resources and capabilities.

Specific objectives are to:

1) Demonstrate, refine, and disseminate efficient supplemental feeding practices for production of tilapia in fertilized ponds, by means of on-farm trials and additional experimentation;
2) Study and standardize a series of incremental stepwise intensification measures which can be used by farmers to the degree that is most appropriate for their particular situation;
3) Perform the Eighth Work Plan Global Experiment as it has been designed by the PD/A CRSP; and
4) Develop an effective administrative presence within the project, for the purpose of coordinating prime-site functions, and to integrate them constructively into regional activities.

Work Plan and Technical Considerations

1) *Characterization of the Research Site*

The Freshwater Aquaculture Center at Central Luzon State University has already been the subject of general site characterization, including a description of the site layout, climate, water and soil quality parameters (Bowman and Clair, 1996; Egna, 1997).

2) *Proposed Collaborative Work*

The body of our proposed research directly addresses one of the priority areas for research activities listed in the Request for Proposals—intensification of aquaculture production techniques. The concept of a stepwise program of intensification options was developed in discussions with farmers and host country scientists. In addition, our response to the host country's request for assistance with problems in the consistency of hatchery production of monosex tilapia will address the first priority, although more as a matter of technical assistance than as a formal program of experimental investigations. We believe the proposed activities present a balance of scientific and technical work that integrates the research interests of the US and Host Country participants, in keeping with point 8 under "Major Programmatic Considerations," which emphasizes recognition of joint decisionmaking between Lead Institution and Host Country PIs.

The program will begin with on-farm trials aimed at demonstration of practices which increase fish production in fertilized ponds by supplemental feeding with lower feed inputs than are required for complete feeding throughout the entire production cycle. Our working hypothesis is that feeding at less than satiation promotes growth cost-effectively, and can provide a means of incrementally increasing the degree of intensification which will be of practical use to farmers.

The work begins on-farm in order to facilitate adoption of successful practices both within and beyond the collaborating group as rapidly as possible. On-station activity and resources will be invoked to answer questions which may arise in management of the on-farm work or in analysis of the data. Because on-farm trials differ from on-station experimentation in terms of the degree to which variables can be controlled, on-farm trials will be used to detect different outcomes resulting from only two very different levels of a factor, daily ration for example. Should outcomes differ substantially, on-station experiments can then examine the factor at several well-controlled levels in order to determine a response curve. In addition, the on-station pond experimentation will contribute to the development of a standardized set of stepwise intensification measures that can be used for pond production systems. The incorporation of less expensive, locally available materials into feeds will be addressed by a collaborating US-based CRSP component project (U. of Arizona) during the effective dates of our project and presumably during its continuation. We will provide technical support for this project and will integrate its results into our own intensification studies. Also, the CRSP Eighth Work Plan Global Experiment will be carried out, in keeping with the guidelines set forth by the Technical Committee.

The proposed work can begin with on-farm trials because the initial values of trial parameters (ration levels, etc.) can be adapted for local conditions from results obtained by the CRSP Thailand project (Diana et al., 1994a, b, c). Technical exchange with the AIT site is given a high priority for our project, not only because the work that we propose follows similar lines of thought, but also because we feel that cooperation between CLSU and AIT will be important in the emergence of the new prime site and in the eventual fulfillment of its role as a center for regional activities.

The program will progress through similar stages of optimization of feeding practices, detailed below. It is fairly safe to predict that each of the conceptual stages will successfully demonstrate improvement (saving of feed inputs and reduced loading of static and output waters) over constant feeding regimes, but with differences particular to the regional socioeconomic and natural environments.

Because this work is oriented to prompt dissemination, an important part of the activity will be production of a sequence of handout pamphlets and presentation aids (overhead computer screen projection, videotape recordings) matched to each of the anticipated stages of technical progress in on-farm trials. Upon completion of each topic in one or more on-farm trials, a farmers' workshop session will be arranged to disseminate the results and practical consequences. Farmer collaborators will be given pamphlets based on results of the previous stage for distribution to visitors to their farms. The involvement of an Aquaculture Extension Specialist from UH at the level of Associate Investigator is intended to improve the quality and efficiency of dissemination of results to farmers.

Experimental Design

The on-farm trials will proceed with a common framework:

Number of Farmers: At least 20 farmers will be enlisted for each trial; no more than two comparison groups per trial will be made. Although yield data will be examined for differences between groups, the primary orientation is to demonstrate the efficacy of practices performed by all participants.

Stocking: Ponds will be stocked with sex-reversed Nile tilapia (*Oreochromis niloticus*) of a single strain for each trial, at an individual weight of 5 to 10 g/fish, and a density of 4 fish/m^2. The best available strain will be chosen based on results of current trials. This density is higher than the optimal 2 to 3 fish/m^2 used for fertilized, unfed ponds, but lower than the observed optimum of approximately 6 fish/m^2 for the Thailand experiments. Density will be adjusted later in the series of trials as described below.

Fertilization: All ponds will be fertilized weekly with urea and 16-20-0 at a rate of 4 kg N/ha/d and an N:P ratio by weight of 5:1, for at least 60 days in each trial. This is done in order to feed the fish from the pond ecosystem as long and as well as possible, testing the input of prepared feed as a supplement.

Sampling: Ponds will be sampled monthly for water quality (water-column integrated samples for analysis of dissolved oxygen, pH, total alkalinity, total ammonia, and soluble reactive phosphorus) to document the nutrient regimes supporting the blooms and which constitute the loading of waters to be discharged upon harvest. Analyses will be done at the FAC laboratory according to standard methods (Boyd, 1979; APHA, 1980). Fish will be sampled monthly for bulk weight and count of at least 25 fish taken without pattern from a seine haul. Ponds will be harvested by seining and complete draining where possible at 120 to 150 days in pond, or at an approximate fish weight of 300 g/fish, a currently desirable "larger" market size.

The sequence of trials will be:

1) *Timing of the Onset of Supplemental Feeding*

Diana et al. (1994b) showed that initiation of feeding of *O. niloticus* after 80 days in the pond produced the same yield at the same time as initiation of feeding at 38 days. Later feeding also increased growth rates and yields to the target levels, but with some delay over earlier feeding. This means that fish do not need to be fed immediately upon stocking, but can be supported by the pond ecosystem. For this on-farm trial, two groups of farmers (all able and willing to buy sufficient feed for the trial) will begin feeding the fish at 30 and 60 days-in-pond. Results may indicate that refinement of the optimal feeding time is possible with an on-station trial which examines more than two periods of delay before feeding.

2) *Reduction of Rations below Satiation Levels*

Diana et al. (1994a) showed that yields of *O. niloticus* were nearly equal at rations of 100%, 75%, and

50% of experimentally determined satiation levels. In other words, reduced amounts of feed may be offered without sacrificing production, but only if these amounts are carefully determined. For this trial, two groups of farmers will feed ponds twice daily at 100 and 67% of experimentally determined satiation. The determination of satiation requirements will be made once per week by project personnel on each farm, who will mark a container for the farmer's use with the volume of feed to be offered twice daily for the coming week. Results may indicate that refinement of the optimal ration will be possible following an on-station trial which examines more than two ration levels.

3) *Stocking Density and Feeding Regime for Optimal Pond Production*
At least 20 farmers will be recruited to participate in a trial which offers a range of input (and likely yield and profit) options, based on a continuum from low feed inputs and densities up to the feeding and stocking parameters representing a fully intensified system. Based on the Thailand results and the design of trials 1 and 2 above, the highest intensity level in this trial would be conducted with initial fertilization before fish stocking at 6 fish/m^2, and supplemental feeding at 67% satiation to begin after 60 days in pond. Lower stocking and input options would be arranged either to match the capabilities and willingness of participants or to create a range of input levels artificially. An extension workshop will explain the trial near its beginning; participants and others will be invited to visit participating farms in the company of project personnel during the trial.

4) *Workshops and Production of Improved Extension Materials*
Each on-farm trial will be analyzed, and a farmers' workshop prepared based on the results. If on-station experimentation is called for, the workshop will not await its completion, but will proceed, with a target date not later than three months after a trial's harvest is completed. Extension will be facilitated by production of illustrated pamphlets describing each of the stages discussed above. The fertilization and feed leaflets used by the AIT projects in Northeast Thailand will serve as a model for development of materials here. These will be refined by circulation of drafts to other organizations (Bureau of Fisheries and Aquatic Resources, BFAR) involved in aquaculture extension functions, and by reviews of draft documents by selected recipients to determine the clarity, effectiveness, and appropriate technical level of the information. Participating farmers will be given multiple copies of the pamphlets appropriate to each completed trial, for distribution to their own neighbors and visitors. The high level of literacy in the Philippines makes distribution of printed materials an effective nationwide mechanism for dissemination.

Expected Outcomes
At the end of the first two-year period, the first two on-farm trials will have been completed, and the collaborators arranged for the third. One or two of the post-trial workshops will have been held. Accordingly, approximately 100 farmers will have had direct contact with project personnel and results, with secondary contacts being much more numerous. Intensification of pond production of tilapia will have been fostered along more efficient and sustainable pathways than would otherwise have been the case if unguided farmers proceeded from opportunistic motives to overstock and overfeed. Sustainability will further be favored by availability of the product of the projected U. of Arizona project, namely, a low-cost supplemental feed based on locally available materials. The extension materials will be available for at least the first trial and workshop, with those for the second near at hand. Project personnel and others will have learned how to use the appropriate tools and materials, which will likely be shared widely at FAC, and will have this project's products as examples.

Beneficiaries
The primary beneficiaries will be the fish producers and consumers of central Luzon, with much wider effect including other regions of the Philippines and Southeast Asia. Yields and parameters, in differing from those obtained on-station in Thailand, will illustrate differences and ranges of outcomes to be expected elsewhere, for example in AITs target area in Vietnam, Laos, and Cambodia.

Other beneficiaries will include other faculty and staff of CLSU, staff of BFAR and other government agencies, and the clientele (students, advisees) of all. CRSP researchers will benefit from the comparisons noted above, and be better able to design and predict outcomes of work in other locations.

Finally, this work has direct applicability to the US tilapia industry because feeds are a major input cost at all levels of system intensification, and all information about the response of fish growth to different feeding practices is valuable when systems are analyzed or designed anew.

Schedule

The first year's work will consist of establishment of the project team, refurbishment of facilities and equipment stocks as necessary, coordination of start-up for collaborating projects, conduct of the first on-farm trial, analysis of its results and preparation of workshop and distribution materials, conduct and evaluation of the first workshop, and initiation of the Eighth Work Plan Global Experiment*. Thereafter, on-station work which may be called for by the results of the on-farm trial will be performed, and at the same time the next on-farm trial will be initiated. The approximate pace of the work will be one trial, one workshop, and on-station work as appropriate to the trials and the Global Experiment, each year. This means that the third on-farm trial will probably take place during a projected third-year period.

Timing of the Onset of Supplemental Feeding (9FFR4)

Start of investigation: January to February 1999
End of investigation: May 1999
Final report due: July 1999

Reduction of Rations below Satiation Levels (9FFR3)

Start: September 1999
End: January 2000
Final report due: July 2000

Workshop and Production of Improved Extension Materials (9ADR6)

Production of improved extension materials completed: October 1999
Start of first workshop: December 1999
End of first workshop: December 1999

Start of second workshop: March 2000
End of second workshop: March 2000

Final report due: July 2000

Eighth Work Plan Global Experiment (8FFR1Ph)

Cool season trial start: December 1999
Cool season trial end: March 2000

Warm season trial start: May 1999
Warm season trial end: August 1999

Final report due: July 2000

Regional Emphasis of the Planned Research

We recognize the need for an active commitment to project coordination and integration on local and international levels. The University of Hawaii will provide a large measure of this form of support to the project. The first administrative priority in our plan will be to increase and simplify communications with the CLSU site, which have been unreliable. The FAC campus is currently not connected to telephone services by wire, although such connections are anticipated in the near future. The lack of a hardwired phone link has made communications by fax, phone, and email difficult; it has been a

* Though the Philippines Global Experiment is printed as part of this work plan, it was funded under Eighth Work Plan research and is in fact the same Global Experiment conducted at other CRSP research sites under the Eighth Work Plan. (As noted in the Introduction to this volume, the Ninth Work Plan does not call for a Global Experiment as a consequence of funding cuts to the program in the third year of the current 5-year grant.)

common experience of PD/A CRSP personnel to send a message to CLSU and wonder for several days if it may have been received. We have also had Express Mail disappear without a trace. A major improvement of communications at the outset of our project is essential if we are to accommodate current and future CRSP projects wishing to arrange site visits or requiring soil and water samples, etc.

Our approach will be to install a dedicated project computer at FAC/CLSU with the best available processing ability and communications software, and subscribe to cellular and Internet service providers, so that project participants can communicate electronically on a more frequent basis, any time they wish. We have contacted available Internet servers in Manila, which offer several apparently workable service options. We will evaluate cellular services in order to obtain the fastest possible data transmission rates, and when phone lines reach the FAC/CLSU site, we will be among the first to obtain a connection.

The strategy of project management and regional integration hinges to some extent on the fostering of cooperation between the CLSU prime site and the former prime site at the Asian Institute of Technology in Thailand. Our vision of the role of CLSU is that of a center from which new technology can radiate throughout Southeast Asia, and as a base for the establishment of future USAID initiatives in other countries able to benefit from enhanced aquaculture development, such as Sri Lanka, Bangladesh, and Nepal.

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Thailand Research

Collaborating Institutions
Asian Institute of Technology
Amrit Bart

The University of Michigan—Lead US Institution
James S. Diana
C. Kwei Lin

Taro-Fish Culture in Ponds: Recycling of Pond Mud Nutrients

New Aquaculture Systems/New Species Research 1 (9NS1)/Experiment

Objectives
1) To assess the pond mud nutrient recovery by taro plants.
2) To assess pond mud characteristics after taro-fish culture.
3) To compare fish growth between with and without taro integration.

Significance
Regular fertilization in fish ponds accumulates nutrients in pond mud. One hectare of old pond mud was reported to have the equivalent of 1.85 tons of urea and 2.30 tons of TSP (Shrestha and Lin, 1996a) or 2.8 tons of urea and 3.0 tons of TSP (Yang and Hu, 1989). Pond muds are major sink for phosphorus and adsorption capacity is related to mineral composition and clay content of pond muds (Shrestha and Lin, 1996b). Release of adsorbed P to water column is minimal and phytoplankton are not as effective to utilize adsorbed P as rooted crops. Roots extended in interstitial water of soil provide better opportunity to extract P from soil (Denny, 1972; Boyd 1982; Smart and Barko, 1985) and hence, pond muds have been widely used to fertilize land crops (Muller, 1978; Little and Muir, 1987; Christensen, 1989; Shrestha and Lin, 1996a). However, removing pond mud is labor intensive and its practicability is questionable (Edwards et al., 1986). Alternatively, taro-fish culture may be considered to utilize reserve nutrient in muds. Taro (*Colocasia esculenta*) is a semiaquatic submerged plant which is seen to grow as tall as one meter. It can utilize adsorbed nutrients from pond sediments efficiently (Shrestha and Lin, 1996a). Water levels of ponds can be increased as taro grows and fish can be stocked. However, taro cultivation in ponds may lead to space competition for fish. Additionally, taro shoots will provide substrate for the growth of epiphytic algae which is consumed by tilapia (Bowen, 1982; Lowe-McConnell, 1982; Shrestha and Knud-Hansen, 1994).

Anticipated Benefits
Results of the experiment will provide information on the possibility of taro-fish culture and recycling of pond mud nutrients which are otherwise wasted. It will generate information on bottom mud characteristics altered by rooted plants. It may benefit small-scale farmers of Asian countries for resource utilization where taro is commonly grown as a root crop.

Research Design
Location: Agriculture University, Nepal (or AIT, if not possible)

Methods: Pond Research

Pond Facility: 9 ponds of 200 m^2 size

Culture Period: 6-7 months

Test Species: Nile tilapia (*Oreochromis niloticus*); taro (*Colocasia esculenta)*

THAILAND

Stocking Density: Tilapia $2/m^2$; taro plant spacing 0.7 x 0.4 m

Nutrient Input: Weekly fertilization by urea and TSP @ 4 kg N and 1 kg $P \cdot ha^{-1} \cdot d^{-1}$

Water Management: After taro planting, water level will be increased as the height of taro plant increases. Once the water level reaches 30 cm, fish will be stocked. Water level will be increased with growth of taro up to 1 m depth.

Sampling Plan: Biweekly and monthly diel water quality following standard CRSP protocol. Initial and final pond mud sampling for organic C, total N, available N, total P, available P, soil pH. Partial budgets will be estimated for cost of inputs and value of fish and taro. Fish growth and survival will only be assessed at the end of the experiment due to sampling difficulties. Fish and taro will be harvested by draining. Nutrient budgets will be estimated for all ponds. We intend to expand our studies into Nepal, and would prefer to conduct this experiment there if possible. If such arrangements cannot be made, we will conduct the study at AIT.

Experimental Design, Hypotheses and Statistical Methods: Experiment will have 3 treatments in triplicates: (a) taro-fish culture, (b) only fish, and (c) only taro. The null hypothesis is that there will be no difference in mud nutrient differences, soil characteristics, fish growth, and nutrient recovery between two treatments. Significant differences will be tested using ANOVA.

Regional Integration
Taro is a popular crop in southeast Asia and other parts of Asia. Nile tilapia is commonly cultured in the region. Small-scale farmers are resource limited and taro-fish culture may utilize waste nutrient resources otherwise.

Schedule
June - December 1999

Report Submission
March 2000

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Culture of Mixed-Sex Nile Tilapia with Predatory Snakehead

New Aquaculture Systems/New Species Research 2 (9NS2)/Experiment

Objectives

1) To assess the efficiency of snakehead in controlling overpopulation of mixed-sex Nile tilapia in ponds.
2) To assess growth and production of Nile tilapia in monoculture and polyculture with snakehead.

Significance

Aquaculture for lower trophic level species such as tilapia presents the greatest potential for efficiency (Welcomme, 1996). However, overpopulation of tilapia in the culture system causes stunting due to shortage of food, which is a problem in tilapia culture. Various methods of population control, such as culture in cages, culture with predators, intermittent harvesting, hybridization, induction of sterility, and production of super male fish (YY-male) have been described (Mair and Little, 1991). However, population control of tilapia through culture with predators is not as well studied. Snakehead (*Channa striata*) has been reported to be used in polyculture with tilapia to keep the population of tilapia under control, or with carps to keep out other extraneous pest fish in the pond system (Wee, 1982). Snakehead swallow their prey whole (Diana et al., 1985), are highly predaceous, and prey on live tilapia fry when provided (Kaewpaitoon, 1992). A population including 5% snakehead with tilapia has been demonstrated to control tilapia recruitment (Balasuriya, 1988). It is unclear how widely such a ratio can be used, since fry production and predator consumption are strongly site-specific. In Thailand, negligible tilapia recruitment is generally found where snakehead was collected from tilapia ponds during harvest, supporting the general concept.

Anticipated Benefits

The results of this study will evaluate an alternative technique for tilapia culture system. Nile tilapia production will expand and increase where sex-reversed tilapia are not available. It will benefit culturists throughout southeast Asia and other tropical countries where tilapia are commonly cultured and there is no tilapia production of sex-reversed fry.

Research Design

Location: AIT, Thailand (or Nepal, if possible)

Methods: Pond research

Pond Facility: 18 earthen ponds, 200 m^2 size

Culture Period: 150 days

Stocking Density: 2 tilapia/m^2; snakehead as specified in treatment design

Test Species: Nile tilapia (*Oreochromis niloticus*); snakehead (*Channa striata*)

Nutrient Input: Weekly chemical fertilization by urea and TSP @ 4 kg N and 1 kg $P \cdot ha^{-1} \cdot d^{-1}$

Water Management: Maintain at 1 m depth

Sampling Plan: Biweekly and monthly diel water quality following standard CRSP protocol, monthly growth and total harvest of fish. Partial budgets will be calculated to estimate input costs and fish value.

Statistical Design, Null Hypothesis and Statistical Analysis: Experiment design will consist of 6 treatments in triplicate:

a) monoculture of sex-reversed tilapia,
b) monoculture of mixed-sex tilapia,
c) polyculture of mixed-sex tilapia and snakehead at 10:1 ratio,

d) polyculture of mixed-sex tilapia and snakehead at 20:1 ratio,
e) polyculture of mixed-sex tilapia and snakehead at 40:1 ratio, and
f) polyculture of mixed-sex tilapia and snakehead at 80:1 ratio.

Stocking size will be about 15 g for tilapia, and a consistent size of snakehead, generally < 5 g.

The null hypotheses are that different ratios of tilapia:snakehead will not have differing effects on tilapia recruitment, and that polyculture or monoculture will have similar tilapia growth and production. Significant differences will be tested using ANOVA.

Regional Integration

Nile tilapia are commonly cultured in Southeast Asia and are introduced in most of the tropical and subtropical Asia. Snakehead are a common indigenous species of tropical and subtropical Asia and are cultured in Thailand.

Schedule

July to November 1999

Report Submission

January 2000

References

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Integrated Recycle Systems for Catfish and Tilapia Culture

Effluents and Pollution Research 3 (9ER3)/Experiment

Objectives

1) To use effluents from intensive catfish ponds as nutrients for tilapia culture ponds, and thus to reduce effluent effects from catfish culture.
2) To gain extra fish production at low cost, making aquaculture more profitable to farmers.

Significance

Clarid catfish has been one of the most popularly cultured freshwater fish in Southeast Asia. The present annual production in Thailand is estimated to be 50,000 tonnes. As an air breather, catfish can be grown at extremely high density (100 fish/m^2) with standing crop in pond culture reaching as high as 100 tonnes/ha (Areerat, 1987). The fish are mainly cultured intensively and fed with trashfish, chicken offal or pelleted feed, which generally causes poor water quality and heavy phytoplankton blooms throughout most of the grow-out period. To maintain the tolerable water quality for fish growth, pond water is exchanged at later stages of the culture cycle (which is 120 to 150 days). The effluents containing concentrated phytoplankton biomass and nutrient, are unsuitable to irrigate rice fields because unbalanced N:P ratios (high nitrogen content) cause rice to fail to fruit. Wastewater disposal from catfish ponds has become a serious problem, especially in the Northeast Thailand where surface waters are in short supply. Farmers often discharge the wastewater to adjacent rice fields, which are damaged by this input. Apparently benefiting from the nutrient rich effluents, aquatic spinach (*Ipomea aquatica*) often grows profusely in those areas. This aquatic macrophyte can be harvested as a vegetable which is widely consumed throughout the region. To fully utilize the effluents, unproductive wetlands can be excavated for tilapia culture and water spinach planted to cover a portion of the pond surface area. Such diversification and integration are regarded as important practices to enhance aquaculture sustainability (Adler et al., 1996; Pillay, 1996).

The wastes from catfish cultured in cages have been shown to be effective for producing phytoplankton to support Nile tilapia culture in the same pond (Lin et al., 1990; Lin and Diana, 1995). Similarly, tilapia reared in cages, feeding on phytoplankton in intensive channel catfish ponds, were shown to improve pond water quality as well as produce an extra crop (Perschbacher, 1995). Although use of plants, such as water hyacinth, to remove nutrient from sewage effluents is done in many areas, few examples have been established for intensive aquaculture in the tropics. Preliminary experiments done at AIT show that water spinach grew rapidly in effluents of domestic wastewater, but the nutrient uptake capacity of this plant yet has to be determined.

Anticipated Benefits

The integrated recycle system will be able to produce tilapia and water spinach using effluents from intensive catfish ponds, which otherwise would be a source of pollution to surface waters. Economically, the profit margin of catfish culture will be augmented with tilapia and water spinach crops at minimal cost. This system will provide scientific information on mass balances of nutrients and optimization of biological productivity.

Research Design

Location: AIT campus, Bangkok

Pond Facility: 15 earthen ponds of 200 m^2 size

Culture Period: 150 days

Stocking Density: 25 catfish/m^2; 2 tilapia/m^2

Test Species: Hybrid catfish (*Clarias macrocephalus* x *C. gariepinus*); Nile tilapia (*Oreochromis niloticus*)

Nutrient Inputs: Pelleted feed for catfish, effluents recirculated to tilapia ponds; tilapia pond will also be fertilized for the first month.

Water Management: Pond water depth to be kept at 1 m; in recirculation treatment the water in catfish ponds will be continuously circulated to tilapia ponds at a rate of one exchange per week. No water circulation will be done in the first month.

Sampling Schedule: Water quality parameters will be analyzed biweekly and diel samples monthly, following standard CRSP protocols. Partial budgets will be estimated to assess costs and value of fish.

Statistical Design and Analysis: The experiment treatments will include catfish alone (control), catfish and tilapia, or catfish plus tilapia and spinach; each treatment will be conducted in triplicate. Ponds will be flooded and stocked with fish. Spinach treatments will have 1/4 of the pond area with bamboo stakes at 3 x 3 m intervals. Spinach will be attached to these stakes. Once spinach reaches full cover, it will be harvested biweekly to reduce crowding. Nutrient budgets will be determined.

Null Hypothesis: Water circulation to tilapia/spinach ponds does not affect water quality and fish production.

Impact Indicators

The experimental results on fish production, economical return and water uses will be compared to average catfish and tilapia production of a select group of Thai farmers in the Bangkok area.

Regional Integration

In the SE Asian region, both clarid catfish and tilapia are widely cultivated with traditional segregated pond culture systems. The integrated systems will be a new step in production technology that will promote efficient production as well as environmental sustainability.

Schedule

15 March to 1 August 1999

Report Submission

15 November 1999

References

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RESEARCH SUPPORT

Preparation of the PD/A CRSP *Continuation Plan 1996-2001* entailed a review of current aquaculture literature and discussions with many aquaculturists to determine research needs and constraints to aquaculture development in developing countries. In addition to limited knowledge of various aspects of production systems, lack of access to training and to information were found to restrict aquaculture development. In response to these needs, the Continuation Plan created research support as a separate building block of its proposed research activities. The following components—Information Management and Networking, Central Database, and Educational Development—are the three branches of the CRSPs research support activities. The prescribed reporting requirements and timelines of the grant form the major part of the work plan of the Information Management and Networking Component which is, therefore, not reported here.

The purpose of the Central Database is to provide a centralized storage, search and retrieval, and analysis system for aquaculture research data generated under the CRSP. For CRSP researchers, the Database (1) provides mechanisms for analysis of variance among geographically dispersed aquaculture research sites, as well as analyses at single locations, and (2) supports the development and parameterization of predictive, simulation models for aquaculture pond processes. In addition, the Database provides a research publication and outreach mechanism for the CRSP that leverages the usefulness of CRSP research to the greater aquaculture community. Publication of complete, standardized, experimental datasets supports users of this information in ways that traditional written publications cannot, ranging from comprehensive, empirical benchmarks of specific fish culture methods to multivariate statistical analyses using combined datasets.

The purpose of the Education Development Component (EDC) is to support human capacity development by providing appropriate education and training opportunities in the countries in which the CRSP works. The EDC works with US and host country Principal Investigators and with host country advisory panels to design and implement appropriate training and education activities that support the research themes at each site.

Database Management

Introduction

The PD/A CRSP Central Database is a centralized storage and retrieval system for aquaculture research data. The Database is open to data submission from all CRSP-sponsored research, as well as other aquaculture research efforts with compatible objectives and compliance with standardized methodology. For data use, the Database is available cost-free and is of interest to researchers, educators, outreach and extension agents, and producers in pond-based aquaculture. Datasets may be searched and retrieved based on user-specified location, calendar year, fish species, and desired types of data. Data searching based on fish culture methods is under development. A comprehensive interface to the datasets and related information in the Database is provided at the Database website <http://biosys.bre.orst.edu/crspDB/default.htm>. This publication mechanism provides immediate and comprehensive access to the Database worldwide.

The Database currently contains over 100 aquaculture production studies and represents the world's largest inventory of standardized aquaculture data. The majority of studies currently in the Database are for production of Nile tilapia (*Oreochromis niloticus*) in sub-tropical and tropical, solar algae ponds, receiving inputs of plant materials, inorganic/organic fertilizers, and/or prepared feeds. Studies of other pond fishes and penaeid shrimp, under monoculture and polyculture management, are also available. Countries with research and research-support projects that have contributed to the Database include Egypt, Honduras, Indonesia, Kenya, Panama, Peru, Philippines, Rwanda, Thailand, and the USA.

Objectives of work to be completed under the Ninth Work Plan address needs of both data suppliers and users. For data suppliers, objectives are to improve the timeliness, efficiency, completeness, and incentives for data submission. For data users, objectives are to improve data and support information access, extraction, and synthesis procedures. These tasks are in addition to the ongoing and primary responsibility of data entry into the Database as datasets are received from researchers.

PD/A CRSP Central Database: Development and Management

Database Management 2 (9DM2)/Activity

Collaborating Institution
Oregon State University
John Bolte
Doug Ernst

Objectives

Objectives of work for the CRSP Database to be completed under the Ninth Work Plan are listed below. This work will be accomplished using a number of Internet and software tools and programs (Ernst et al., 1997). Tasks to be completed will address needs of both data suppliers and users.

1) Continue to add new datasets to the Database as they are submitted. This will be accomplished with rigorous quality control measures so that problems with the Database as it was inherited by its current management (May 1996) will not be repeated.
2) Continue to improve the integrity of data in the Database from past data submissions. This sizable task was not anticipated in the Eighth Work Plan and continues into the Ninth Work Plan.
3) Provide incentives for aquaculture research groups to submit data to the Database, both within and external to the CRSP. Two major components of this task are to make it as simple and efficient as possible to submit data to the Database while also making it professionally rewarding to do so. In support of the latter, automated author citations for extracted datasets will be made available to data users, and use of the Database will continue to be promoted to the aquaculture community through the Internet and professional meetings.
4) Complete the development of experiment treatment protocols (research materials and methods) for all existing data in the Database and require this information for future data submission. This information is critical to data users for both identifying specific datasets and for understanding the context under which datasets were generated. Experimental methods used by researchers are equivalent to the alternative methods used by fish culturists and represent the basis of user-defined data queries. In cooperation with the CRSP Technical Progress Subcommittee, a past and current projects section will be added to the Database web site. This section will support the entry of entirely new studies, as well as materials and methods for existing studies.
5) Continue development of standardized methods and data templates for economic and socio-economic type data in conjunction with CRSP expertise in this area.
6) Enhance the Database web site with supporting information. This includes 1) Data submission manual, 2) Data user's manual, 3) PD/A CRSP Handbook of Analytical Methods, 4) Work Plan summary table, 5) Study publications and authors, and 6) context-sensitive links to site and facility, publication, and author information maintained at the CRSP Program Management Office. This work is being accomplished in collaboration with the CRSP webmaster.
7) Explore alternative interface programming mechanisms for the Database web site, specifically client-based (Java, Sun Microsystems) versus server-based (Cold Fusion, Allaire) procedures. The objective of this work is to provide Database web site users with the best possible interface to the Database under available computer technology.
8) Develop automated mechanisms to generate experimental-treatment summary statistics for data users. This will consist of summarizing treatment replicates by the use of range, mean, and variance statistics. In addition to reporting purposes, these statistics will also be used to compare treatments based on analysis of variance. For many data users, these synthesized data will be much more valuable than the raw, treatment-replicate data that are now provided.
9) Develop automated regression procedures to determine equation coefficients for fish growth functions (linear, exponential, and sigmoidal) using independent variables of water temperature, food resource availability (endogenous productivity and supplemental feeding rate), and fish weight. These regression parameters and associated fish growth models will provide design and planning tools for pond-based aquaculture.

10) Develop an automated mechanism to summarize all experimental data in the Database to a single table, organized by experimental treatment. This table will be made available to aquaculture database collaborators on an annual basis (e.g., International Center for Living Aquatic Resource Management, ICLARM).

Significance

Two fundamental rationales for the original establishment of the CRSP Database were to:

1) Create a mechanism for analysis of variance among geographically dispersed aquaculture research sites, in addition to analyses within single ponds and among ponds at a single location, and
2) Support development of predictive models for aquaculture pond processes (Egna et al., 1987).

The purpose of the work proposed here is to both continue and expand this effort.

Availability of standardized aquaculture information as computerized database files is an invaluable resource for aquaculture planning, design, and management (e.g., Batterson et al., 1991; Froese and Pauly, 1996; Pedini and Coppola, 1996; Network of Aquaculture Centres in Asia-Pacific, Bangkok, Thailand). Information related to aquaculture is similarly useful (e.g., Consortium of International Earth Science Information Networks; USAID Tropsoils CRSP; and the Small Water Body Database, SADC/ALCOM, Harare, Zimbabwe). Such databases serve as a centralized repository for multiple studies and help alleviate literature search and retrieval tasks. In addition, datasets are immediately available for computerized statistical analyses and parameterization of aquaculture process models.

The Database provides a unique and valuable research-outreach mechanism for the PD/A CRSP (Ernst et al., 1997). The Database provides a model for standardized design and reporting of pond-based aquaculture research, and it provides a publication mechanism that leverages the usefulness of aquaculture research to the greater aquaculture community. Publication of complete, standardized, experimental datasets supports the users of this information in ways that traditional written publications cannot, ranging from unobstructed perspectives on empirical fish production to multivariate statistical analyses (Prein et al., 1993).

Anticipated Benefits

In order for information generated by aquaculture research to support advancements in production practices, it must be published in easily accessible and useful forms. The CRSP Database directly addresses this need by serving as an information conduit from aquaculture research to aquaculture practice. The primary benefit of the proposed work is an enhanced capacity of the aquaculture community to utilize the information generated by aquaculture research. The anticipated impact following from this, given that research studies contained in the Database address real user needs, is that this work will further the development of efficient and sustainable aquaculture practices. Finally, the Database provides a worldwide and world-class model for standardized design and reporting of pond-based aquaculture research. These standardized research and reporting methods provide the essential foundation for efficient and accurate interpretation and application of aquaculture research.

Activity Plan and Additional Items

The CRSP Database currently resides on a Windows-NT server and is maintained using relational database software (Access, Microsoft). A server application (Cold Fusion, Allaire) is used to support client-server database access and database publication via the Internet (World Wide Web). A number of web forms have been developed to support tabular data retrieval. A programming language (Java, Sun Microsystems) is used to embed time-series and water-depth-based plots in web pages for graphical data retrieval.

The Database can be accessed free of cost by aquaculture researchers, educators, outreach and extension agents, and producers. Data may be searched and extracted according to geographical site, calendar year, fish species, and fish production methods, the latter to be completed under the Ninth Work Plan. An interface to the Database is provided at its Internet web site, located at <http://biosys.bre.orst.edu/crspDB/default.htm>. This publication mechanism provides immediate and comprehensive access to the Database worldwide.

The location of the work will be the Department of Bioresource Engineering, Oregon State University, Corvallis, OR USA. The methods by which this work will be carried out are either stated under the objectives above or are too detailed for the scope of this document.

Regional Integration

The Database maintains datasets from all sites and will continue to work with all regions to improve and enhance the quality of the Database. Efforts will be distributed evenly across the four designated CRSP regions.

Task Schedule

References

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Prein, M., G. Hulata, and D. Pauly, (Editors), 1993. Multivariate Methods in Aquaculture Research: Case Studies of Tilapias in Experimental and Commercial Systems. Std. Rev. 20, ICLARM, Manila, Philippines, 221 pp.

Education Development Component

Introduction

The purpose of the Education Development Component (EDC) is to strengthen human capacity by providing appropriate education and training opportunities in the countries in which the CRSP works. During the Eighth Work Plan, education advisory panels were established in Honduras and the Philippines to work with US and host country Principal Investigators and the EDC to develop an appropriate training plan for each project. Under the Ninth Work Plan, this work will be carried forward via funding of masters-level students in Honduras and the Philippines. This support will help increase research capacity in the host countries by improving the ability of host-country partners to design and implement research projects.

Building Research Capacity in CRSP Host Countries*

Human Capacity Development 2 (9HCD2)/Activity

Collaborating Institution
Oregon State University
Marion McNamara

Cooperating Institutions
Central Luzon State University
Remedios Bolivar

Secretaría de Agricultura y Ganadería, Republic of Honduras
Marco Polo Micheletti Bain

Objectives

1) To build aquacultural research capacity in CRSP host countries.
2) To increase local capacity to direct and manage research programs.
3) To improve communication between research station and extension personnel in CRSP host countries.

Significance
Allocation of sufficient human and financial resources to research and development for improved management is critical to the future of fisheries and aquaculture. US and host country CRSP researchers in Honduras and Philippines agree that the lack of university training inhibits the development of host country aquaculture research programs. As the CRSP moves away from a development model that relies on US expatriates to provide leadership in developing and implementing the research program, it becomes increasingly important to provide opportunities for higher education and training to develop host country capacities to continue the work beyond the life of the CRSP. This activity proposes to support graduate level studies for host country personnel.

Anticipated Benefits
The proposed activity is anticipated to have the following benefits:

1) Improve the knowledge, skills, and abilities of CRSP Host Country researchers to conduct aquaculture research and to communicate results to extension personnel.
2) Increase the cadre of professionals trained to design and implement an aquaculture research program.
3) Improve the long-term sustainability of aquaculture in Honduras and Philippines.

Activity Plan
Masters level students will be supported in Honduras. In the Philippines, masters and doctoral students will receive thesis support stipends for topics related to CRSP research. In each country, an Education Advisory Panel will work with the EDC Coordinator and the US and Host Country PIs to establish the highest priority research area, to recommend most appropriate institutional placement, and to develop criteria for selection of candidates for financial support. Training may take place at appropriate regional or host country institutions of higher learning, or at US institutions, if appropriate. If possible, the US PI and/or HC PI will serve on the sponsored student's graduate committee. The student's research project will address constraints to aquaculture in her/his home country, and research will be conducted in the home country. The EDC will coordinate admission to US universities, and will be responsible for collecting participant data.

The Honduras Advisory Panel has recommended supporting a masters level student at Auburn University. The process of candidate selection will begin in the spring of 1998, and the student will

* As of the time of printing, the PMO has approved this investigation for funding, but the formal subcontract for work is still in process.

begin studies in the spring of 1999. (Note: funding for three-fourths of this activity was included in the Eighth Work Plan.) The Philippines Advisory Panel will be formed during this work plan time period. Initial input from the PIs on the Philippines project suggests that sufficient in-country educational resources exist to provide most of the degree programs needed to prepare students to assume responsibility for aquaculture research in their countries; thus the EDC may be able to support students attending local or regional universities. In the Philippines, masters and Ph.D. students (total of three per year) will receive stipends to support their research on CRSP-related topics.

Schedule

Report Submissions

Students will submit their academic enrollment and quarterly progress reports signed by their professor to the EDC. The EDC will report student progress to the PMO in quarterly and annual reports.

Institutionalizing EDC Activities in CRSP Host Countries*

Human Capacity Development 3 (9HCD3)/Activity

Collaborating Institution
Oregon State University
Marion McNamara

Cooperating Institutions
Central Luzon State University
Remedios Bolivar

Secretaría de Agricultura y Ganadería, Republic of Honduras
Marco Polo Micheletti Bain

Objective
To establish an Education Advisory Committee in each region in which the CRSP works.

Significance
Training and education resources can be optimized by supporting the most appropriate training activities in each region. Government agencies, producers' groups, educational institutions, and NGOs have a stake in the success of aquaculture in each country, and can provide a valuable perspective in determining the training and education priorities for their region. By inviting participation from the stakeholders in the planning stage, the EDC can foster collaboration in the implementation of training activities at each site. Such collaborative efforts will increase chances for the activities success, and will help create a support system for future education and training efforts undertaken by any of the cooperating institutions.

Activity Plan
Education Advisory Panels in each country will consist of the US and Host Country PIs and representatives of institutions that have a stake in aquaculture development in the country or region. The panel will provide guidance to the EDC Coordinator in establishing long-term training and education goals for the country or region. The EDC Coordinator will meet with the panel to discuss long-term goals and strategies for reaching these goals. The EDC coordinator will prepare a written report to the panel outlining the agreed-upon goals and suggesting activities to be undertaken to meet these goals. Panel members will provide evaluative feedback to the EDC Coordinator on the outcomes of training and education activities undertaken. The establishment of these advisory panels will be staged, beginning with Honduras (established in 1997), then the Philippines.

Schedule

Reports
The EDC will provide a report to the committee and to the PMO within 30 days of the initial meeting and within 30 days of each subsequent meeting.

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